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(71) **Applicant** *(for all designated States except US)*: **NANOGEN, INC.** [US/US]; 10398 Pacific Center Court, San Diego, California 92121 (US).

(72) **Inventors; and**

(75) **Inventors/Applicants** *(for US only)*: **SMOLKO, Daniel, D.** [US/US]; 20555 Bee Valley Road, Jamul, California 91935 (US). **SWANSON, Paul, D.** [US/US]; 10854 Susie Lane, Santee, California 92071 (US). **HODKO, Dalibor** [US/US]; 13334 Gem Court, Poway, California 92064 (US). **CANTER, David** [US/US]; 9135 Kensett Place, San Diego, California 92126 (US). **HAIGIS, Robert, W.** [US/US]; 3833 Ingraham St., #A306, San Diego, California 92109 (US). **PATTERSON, Tricia** [US/US]; 3895 Riviera Drive, Apt. A, San Diego, California 92109 (US).

(54) **Title:** METHODS AND MATERIALS FOR OPTIMIZATION OF ELECTRONIC TRANSPORTATION AND HYBRIDIZATION REACTIONS

(57) **Abstract:** Methods for the transport and hybridization of a nucleic acid on an electrode device by providing a low conductivity buffer with a reducing agent to the device. The low conductivity buffer may also contain a zwitterion. A current and voltage is applied to a location of the device to effect electrophoretic transportation of the nucleic acid towards the location. The nucleic acid is then hybridized to a nucleic probed located at the location. The reducing agent in the low conductivity buffer may also be acting as a chaotropic agent.

DESCRIPTION

METHODS AND MATERIALS FOR OPTIMIZATION OF ELECTRONIC

TRANSPORTATION AND HYBRIDIZATION REACTIONS

[0001]   This application is an international filing based on U.S. Application Serial

No. [Attorney Docket No. 612,404-443], filed on September 22, 2005, which claims

the benefit of provisional U.S. Application Serial No. 60/613,148, filed on

September 23, 2004, the entirety of which is hereby incorporated by reference in its

entirety.

Field of the Invention

[0002]   This invention relates to buffers and electrolytes and methods for their use in

electronic devices adapted for medical diagnostic, biological, and other microfluidic

applications. More particularly, it relates to buffers, electrolytes, and methods for their use

with DNA transport and subsequent hybridization analysis carried out on microelectronic

diagnostic devices.

Background of the Invention

[0003]   Recently, there has been increasing interest in devices that combine electronics

and molecular mechanisms. These systems are able to perform a wide variety of functions

that are advantageously used in molecular biology reactions, such as nucleic acid

hybridizations, antibody/antigen reactions, clinical diagnostics, and biopolymer synthesis.

These systems include any electrode device. The system may have a single electrode or

multiple electrodes. One such system is disclosed in "ACTIVE PROGRAMMABLE

ELECTRONIC DEVICES FOR MOLECULAR BIOLOGICAL ANALYSIS AND

DIAGNOSTICS," Serial No. 08/146,504, filed November 1, 1993, now issued as U.S.

Patent No. 5,605,662, which is expressly incorporated herein by reference in its entirety.

[0004]   Electrode devices utilize buffers and electrolytes for their operation. A buffer

has been defined as a chemical solution that is resistant to change in pH on the addition of

acid or alkali. See., e.g., Dictionary of Biotechnology, Second Edition, James Coombs,

Stockton Press. As stated there, "traditionally, buffers based on inorganic salts

(phosphate, carbonate) and organic acid salts (acetate, citrate, succinate, glycine, maleate,

barbiturates, etc.) were used in biological experiments."

[0005]   It is the object of this invention to discover buffers and electrolytes that are

advantageously used in molecular electronic devices that perform transport,

hybridizations, reactions, diagnostics, and/or synthesis.

Summary of the Invention

[0006]   The following inventions relate to our discoveries concerning the various

parameters, electrolytes (buffers), and other conditions that improve or optimize the speed

of transport of charged biomolecules (e.g., DNA, RNA, etc.), the efficiency of

hybridization reactions, and the overall hybridization specificity in microelectronic chips

and devices. In particular, this invention relates to the discovery that low conductance

zwitterionic buffers containing a reducing agent provide optimal conditions for both rapid

DNA transport and efficient hybridization reactions.

[0007]   The method of the present invention relates to transporting and hybridizing

nucleic acids in an electrode device. A low conductivity, zwitterionic buffer containing

a reducing agent is provided to the electrode device. The nucleic acid is then

electrophoretically transported to a location of the electrode device by applying a current and voltage to the electrode at the location. The nucleic acid can then be hybridized to a probe located at the electrode, whereby the local pH above the microlocation is below the pH of the buffer at its isoelectric point. Alternatively, the nucleic acid can be coupled to a permeation layer associated with the electrode at the location.

[0008] In another embodiment of the present invention, the method relates to the transport and hybridization of nucleic acids on an electrode device, wherein the device has a location having a nucleic acid probe. A low conductivity, zwitterionic buffer containing a reducing agent is applied to the electrode device. A target nucleic acid is then applied to the device. The target nucleic acid is then concentrated to the location by applying current and potential to the device. Once at the location, the target nucleic acid hybridizes with the nucleic acid probe.

[0009] In another embodiment of the present invention, the method relates to electronically enhancing hybridization of a DNA analyte to a single stranded capture DNA at a location in an electric field at a positively biased location on an electrode device. A buffer containing a reducing agent and a low conductance, zwitterionic molecule is applied to the device. Current is then applied to the location in an amount sufficient to produce an electric field at the location, wherein the location is positively biased relative to the DNA analyte. As a result, the DNA analyte is transported to the location. Once at the location, the DNA analyte is hybridized to the single stranded capture DNA, wherein positively charged buffer molecules at the location stabilize the hybridization.

[0010] In another embodiment of the present invention, the method relates to transporting a nucleic acid in an electrode device. A double-stranded nucleic acid having a first and a second strand is added to a low conductivity, zwitterionic buffer with a reducing agent. The double-stranded nucleic acid is then denatured by the low conductivity, zwitterionic buffer containing a reducing agent into the first and second single strands. The nucleic acids are then loaded onto the device in the low conductivity, zwitterionic buffer containing a reducing agent. An electrode of the electrode device is also provided with a capture sequence, wherein the capture sequence is complementary to the first strand of the double-stranded nucleic acid. Current and voltage are then applied to the electrode at a temperature of less than about 30°C in order to effect electrophoretic transportation of the first strand towards the electrode. Subsequently, the first strand of the nucleic acid hybridizes with the capture sequence associated with the electrode. In alternative embodiments, the current and voltage are applied to the electrode at a temperature of less than about 28°C, alternatively less than about 25°C, alternatively less than about 23°C, alternatively less than about 20°C, alternatively less than about 18°C, alternatively less than about 15°C, alternatively less than about 10°C.

[0011] In another embodiment of the present invention, the method of the present invention relates to transporting and hybridizing nucleic acids in an electrode device. A low conductivity buffer containing a reducing agent is provided to the electrode device. The nucleic acid is then electrophoretically transported to a location of the electrode device by applying a current and voltage to the electrode at the location. The nucleic acid can then be hybridized to a probe located at the electrode. Alternatively, the

nucleic acid can be coupled to a permeation layer associated with the electrode at the location.

[0012]   In all of the various methods described above, the reducing agent may be α-thioglycerol, dithiotreitol, β-mercaptoethanol, cysteine, or combinations thereof. The concentration of the reducing agent should be sufficient to reduce bubbling at the electrode so as not to interfere with the hybridization of the DNA. The concentration of the reducing agent may be about 25-700 mM, alternatively about 25-500 mM, alternatively about 25 mM, alternatively about 50 mM, alternatively about 100mM, alternatively about 125 mM, alternatively about 150 mM, alternatively about 250 mM, alternatively about 500 mM.

[0013]   In all of the various methods described above, the low conductance, zwitterionic buffer may contain histidine (either D- or L-histidine), γ-aminobutyric acid (GABA), alanine, lysine, glutamic acid, any other naturally occurring or synthetic zwitterion, or any combinations thereof.

Brief Description of the Figures

[0014]   FIG. 1. is the cross-section of three self-addressable microlocations.

[0015]   FIG. 2 is the cross-section of a microlocation.

[0016]   FIG. 3 shows a graphical representation of histidine stabilization of hybrids in the electronic hybridization process.

[0017]   FIG. 4 shows a graph of oxidation/reduction potential sweeps.

[0018]   FIG. 5 shows the products of water and α-thioglycerol oxidation.

[0019]   FIG. 6 is a mass spectrum of an electrolyzed histidine buffer.

[0020]   FIG. 7 is a mass spectrum of an electrolyzed histidine buffer containing α-thioglycerol.

[0021]   FIG. 8 is a mass spectrum of a histidine buffer containing α-thioglycerol.

[0022]   FIG. 9 is a graph of signal accumulation on an electrode device using different buffers.

Detailed Description of the Invention

[0023]   The devices and the related methodologies of this invention allow molecular biology and diagnostic reactions to be carried out under "complete electronic control." The meaning of "electronic control" as referred to in this invention goes beyond the conventional connotation of the term. Most conventional electronic devices, instruments, and detector systems are always at some level under electronic control. The electronic devices of this invention are not only under conventional electronic control, but more importantly, they also provide further direct electronic control over the physical aspects of carrying out molecular biological and diagnostic reactions. This invention provides an electronic device with an electrode. The electronic device may have a single electrode or multiple electrodes. For instance, the electronic device may have 1, 4, 8, 6, 16, 25, 36, 100, 400, 1000, or 10,000 electrodes.

[0024]   One such device is the APEX system. The details of the APEX device are in U.S. Application Serial No. 08/146,504, now issued as U.S. Patent No. 5,605,662, which is referred to and incorporated by reference above. Briefly, FIG. 1 shows a basic design of self-addressable microlocations fabricated using microlithographic techniques. The three microlocations (10) (ML-1, ML-2, ML-3) are formed on the surface of metal sites (12) that have been deposited on an insulator layer/base material. The metal sites (12)

serve as the underlying microelectrode structures (10). An insulator material separates

the metal sites (12) from each other. Insulator materials include, but are not limited to,

silicon dioxide, glass, resist, rubber, plastic, or ceramic materials.

[0025]   FIG. 2 shows the basic features of an individual microlocation (10) formed

on a microlithographically produced metal site (12). The addressable microlocation is

formed on the electrode/metal site (12), and incorporates a permeation layer (22). The

electrode/metal site may include platinum, platinum-silicide, carbon, and any other

suitable material known to one of ordinary skill in the art. The permeation layer

provides spacing between the metal surface and the probes or specific binding entities

and allows for solvent molecules, small counter-ions, and gases to freely pass to and

from the surface of the electrode. Probes or specific binding entities can couple to

attachment sites in the permeation layer. Details of various permeation layers can be

found in U.S. Application Serial Nos. 10/014,895, filed December 10, 2001;

09/464,670, filed December 15, 1999, now issued as U.S. Patent No. 6,303,082; and

09/922/349, filed August 3, 2001, all of which are herein expressly incorporated by

reference in their entirety.

[0026]   After the initial fabrication of the basic microelectronic structure, the device

is able to self-direct the addressing of each specific microlocation with specific binding

entities or capture probes. Thus, the devices and methods of this invention can be

combined into an instrument system that allows addressing of an APEX chip device

with any DNA or RNA probe, or any other ligand. Such a system allows "make your

own chip" products and applications. Such products and applications would be useful

to many researchers and end users for clinical diagnostic, molecular biology, functional

genomic and drug discovery applications. The self-addressed device is subsequently

able to actively carry out individual multi-step and combinatorial reactions at any of its microlocations. The device is able to carry out multiplex reactions, but with the important advantage that each reaction occurs at the equivalent of a truly independent test site. The device is able to electronically direct and control the rapid movement and concentration of analytes and reactants to or from any of its microlocations. The ability of the device to electronically control the dynamic aspects of various reactions provides a number of new mechanisms and important advantages and improvements.

[0027]   There are various physical parameters that relate to the electrophoretic transport of DNA and other charged analytes in various types of electrolyte/buffer solutions. Certain of the devices, e.g., the APEX device as described in Serial No. 08/146,504, referenced above, are primarily DC (direct current) electrical devices. Electrophoretic transport of charged molecules occurs between oppositely (+/-) biased microlocations on the device surface.

[0028]   APEX type devices produce significant net direct current (DC) flow when a voltage is applied, which is recognized as "the signature of electrophoresis." In electrophoresis, the migration of ions or charged particles is produced by electrical forces along the direction of the electric field gradient, and the relationship of current and voltage are important to this technology. The electrophoretic migration shows itself macroscopically as the conduction of electric current in a solution under the influence of an applied voltage and follows Ohm's law.

$$V = R \times I$$

V is the electric potential

R is the electric resistance of the electrolyte $[V \times A^{-1} = R(\Omega)]$

I is the electric current [A].

[0029]   The resistance of the solution is the reciprocal of the conductance, which can be measured by a conductometer. The conductance depends mainly on the ionic species of the buffer/electrolytes and their concentration; therefore these parameters are very important for electric field related molecular biology technology. The basic current/voltage relationships are essentially the same for the APEX technology as for any other electrophoretic system, although the electric fields produced are in truly microscopic environments.

[0030]   There are unique features of the APEX system regarding the various ways of sourcing the current and voltage, and how the current and voltage scenarios have been found to improve the performance of our systems. In particular, various DC pulsing procedures (linear and logarithmic gradients) appear to provide improved hybridization stringency.

[0031]   The details of complexity reduction devices are in U.S. Application Serial No. 08/709,358, now issued as U.S. Patent No. 6,129,828, which is hereby expressly incorporated by reference in its entirety.

Electrophoretic Transport Versus Ionic Strength

[0032]   It is well established in the field of electrophoresis that there is a logarithmic decrease in the mobility of the charged analyte species (proteins, DNA, etc.), which is inversely proportional to the square root of the ionic strength of the electrolyte solution (see page 83 and Fig. 3.16 in "Capillary Electrophoresis: Principles and Practice", R. Kuhn and S. Hoffstetter, Springer-Verlag, 1993). At any given constant electric field strength, as the electrolyte concentration decreases relative to the analyte species (protein, DNA, etc.), the analyte will be transported at a faster rate. Similar results demonstrating

this effect for a danyslated amino acid have been shown by J.J. Issaq et. al.,

Chromatographia Vol. 32, #3/4, August 1991, pages 155 to 161 (see in particular Fig. 3 on

page 157). Results demonstrating this effect for DNA in different electrolyte solutions has

been shown in P.D. Ross and R.L. Scruggs, Biopolymers Vol. 2, pages 231 to 236, 1964

(see in particular Fig. 1, page 232).

Ionic Strength/Conductance Relationship

[0033]   For those non-buffering electrolytes (sodium chloride, potassium chloride, etc.)

that involve completely dissociated anion and cation species in solution ($Na^+ \leftrightarrow Cl^-$, $K^+ \leftrightarrow$

Cl^-, etc.), the ionic strength and conductance are equivalent, i.e., the conductance will

usually be proportional to the ionic strength. For those buffering electrolytes (phosphate,

acetate, citrate, succinate, etc.) that are in their dissociated states (example: $2 Na^+ \leftrightarrow$

PO_4^{-2}), the ionic strength and conductance will usually be equivalent, i.e., conductance is

proportional to the ionic strength. For those buffering electrolytes (Good Buffers - MOPS,

HEPES, TAPS, Tricine, Bicine, Amino Acid Buffers, Ampholytes, etc.) that can have a

zwitterionic species (*no net charge at their pI*), the conductance will decrease by

approximately a factor of 10 for every pH unit difference between the isoelectric point (pI)

and the (pKa). For example, an amino acid in its zwitterionic state ($^-OOC\text{-}CH(R)\text{-}NH_3^+$)

will have a conductance value that will be approximately 1000 fold lower than when the

"*amino acid moiety*" has a full net positive charge ($HOOC\text{-}CH(R)\text{-}NH_2^+ X^-$), or a full

negative charge ($Y^+ {}^-OOC\text{-}CH(R)\text{-}NH_2$). Thus, a formal negative or positive charge

develops on the amino acid moiety as it moves away from its pI, and the conductivity and

ionic strength will begin to correlate. When at or near the pI, however, the conductance

will be much lower than is expected for that given ionic strength or concentration. When

used at or near their pI's, electrophoresis texts refer to the Good Buffers and amino acid buffers as having "low conductance's at high ionic strength or concentration" (see page 88 of Capillary Electrophoresis: Principles and Practice", R. Kuhn and S. Hoffstetter, Springer - Verlag, 1993). A commonly used electrophoresis buffer "Tris-Borate" actually has a significantly lower conductivity than would be expected from its ionic strength or concentration. This may be due to the "tris cation" and "borate anion" forming a relatively stable zwitterionic complex in solution. The conductivity of a 100 mM Tris-Borate solution was determined to be 694 μS/cm, which is approximately 20 times lower than would be expected from its ionic strength, and is roughly equivalent to a 5 mM sodium phosphate or sodium chloride solution. Table 1 shows conductivity measurements of a number of transport buffers.

TABLE 1

Solution/Buffer	Measurement 1	Measurement 2	Measurement 3	Average/Std. Deviation
10 mM MgCl$_2$	1.95 mS/cm	2.02 mS/cm	2.13 mS/cm	2.03+/-0.09 mS/cm
1 mM MgCl$_2$	174 μS/cm	208 μS/cm	177 μS/cm	186+/-18.8 μS/cm
0.1 mM MgCl$_2$	16.9 μS/cm	16.7 μS/cm	18.3 μS/cm	17.3+/-0.87 μS/cm
10 mM NaCl	1.07 mS/cm	1.10 mS/cm	1.18 mS/cm	1.12+/-0.057 mS/cm
1 mM NaCl	112 μS/cm	115 μS/cm	111 μS/cm	112.7+/-2.08 μS/cm
0.1 mM NaCl	8.80 μS/cm	8.98 μS/cm	10.5 μS/cm	9.43+/-0.93 μS/cm
20 mM NaPO$_4$	2.90 mS/cm	2.79 mS/cm	3.00 mS/cm	2.90+/-0.11 mS/cm
10 mM NaPO$_4$	1.40 mS/cm	1.44 mS/cm	1.48 mS/cm	1.44+/-0.04 mS/cm
1 mM NaPO$_4$	122 μS/cm	128 μS/cm	136 μS/cm	128.7+/-7.0 μS/cm
50 mM TRIS	3.50 mS/cm	3.14 mS/cm	3.40 mS/cm	3.35+/-0.19 mS/cm
10 mM TRIS	572 μS/cm	562 μS/cm	583 μS/cm	572+/-10.5 μS/cm
250 mM HEPES	141 μS/cm	144 μS/cm	158 μS/cm	147.6+/-9.07 μS/cm
25 mM HEPES	9.16 μS/cm	9.44 μS/cm	10.5 μS/cm	9.7+/-0.71 μS/cm
3.3 mM NaCitrate	964 μS/cm	964 μS/cm	1.03 mS/cm	986+/-38.1 μS/cm
5 mM NaSuccinate	1.05 mS/cm	960 μS/cm	1.01 mS/cm	1.01+/-0.045 mS/cm
5 mM NaOxalate	1.02 mS/cm	1.03 mS/cm	1.12 mS/cm	1.06+/-0.055 mS/cm
10 mM NaAcetate	901 μS/cm	917 μS/cm	983 μS/cm	934+/-43.5 μS/cm
250 mM Cysteine	27.4 μS/cm	17.3 μS/cm	23.5 μS/cm	22.7+/-5.09 μS/cm
Milli-Q water	<0.5 μS/cm			Detection limit of 0.1 cell too low

Zwitterionic Buffers/Conductance/Transport Rate

[0034]   Certain advantages exist regarding the rate or speed of electrophoretic transport

of DNA when using Zwitterionic buffers (Good buffers, amino acid buffers), or the Tris-

Borate buffer at or near their pI's. These advantages include: 1) zwitterionic buffers can

be used at relatively high concentrations to increase buffering capacity; 2) the conductance

of zwitterionic buffers is significantly lower than other types of buffers at the same

concentration, and 3) zwitterionic buffers have higher electrophoretic transport rates for

the analyte of interest (e.g., DNA, RNA).

Zwitterionic Buffer Capacity at the Isoelectric Point (pI)

[0035] Amino acid buffers have buffer properties at their pI's. While a given amino acid may or may not have its "highest buffering capacity" at its pI, it will have some degree of buffering capacity. Buffer capacity decreases by a factor of 10 for every pH unit difference between the pI and the pKa; those amino acids with three ionizable groups (histidine, cysteine, lysine, glutamic acid, aspartic acid, etc.) generally have higher buffering capacities at their pI's than those amino acids with only two ionizable groups (glycine, alanine, leucine, etc.). For example, histidine (pI = 7.47), lysine (pI=9.74), and glutamic acid (pI=3.22), all have relatively good buffering capacity at their pI's relative to alanine or glycine, which have relatively low buffering capacities at their pI's (see A.L. Lehninger, Biochemistry, 2ed, Worth Publishers, New York, 1975; in particular Fig. 4-8 on page 79, and Fig. 4-9 on page 80). Histidine has been proposed as a buffer for use in gel electrophoresis, see, e.g., U.S. Patent 4,936,963, but hybridization is not performed in such systems. Cysteine is in a more intermediate position, with regard to buffering capacity. The pI of cysteine is 5.02, the pKa for the α-carboxyl group is 1.71, the pKa for the sulfhydryl is 8.33, and the pKa for α amino group is 10.78. An acid /base titration curve of 250 mM cysteine, shows that cysteine has a better "buffering capacity" at ~ pH 5 than a 20 mM sodium phosphate. In the pH 4 to 6 range, the buffering capacity of cysteine is significantly better than 20 mM sodium phosphate, particularly at the higher pH. In these pH ranges, however, the conductance of the 250 mM cysteine solution is very low ~23 μS/cm, compared to 20 mM sodium phosphate, which has a value of ~2.9 mS/cm (a factor of 100 times greater). Low conductance zwitterionic buffers suitable for APEX devices have been disclosed in U.S. Application Serial Nos. 09/986,065, filed December 5, 1997, now issued as U.S. Patent No. 6,051,380; 09/444,539, filed November

22, 1999, now issued as U.S. Patent No. 6,518,022; 10/170,172, filed June 11, 2002; and

08/708,262, filed September 6, 1996, now abandoned, all of which are herein expressly

incorporated by reference in their entirety.

[0036] Several electrophoretic techniques developed over 20 years ago are based on

the ability to separate proteins in zwitterionic buffers "at their pI's," these techniques are

called Isoelectrophoresis, Isotachophoresis, and Electrofocusing (see chapters 3 and 4 in

"Gel Electrophoresis of Proteins: A Practical Approach" Edited by B.D. Hames & D.

Rickwood, IRL Press 1981). Various amino acid buffers and Good buffers were used for

these applications, all at their pI's (see Table 2, page 168 of the above reference).

DNA Transport in Low Ionic Strength and Low Conductance Buffers

[0037] A series of fluorescent checkerboard experiments were carried out using 2.5%

agarose coated 5580 chips and the ByTr-RCA5 fluorescent probe. Rapid (6 second)

checkerboard addressing was achieved in all of the following systems: (1) 250 mM

HEPES (low conductance), (2) 10 μM sodium succinate, (3) 10 μM sodium citrate, and

(4) distilled water. While some types of low conductance or low ionic strength solutions

may have somewhat better characteristics, checkerboard addressing and rapid DNA

transport (6 to 12 second DNA accumulation on an 80 μm pad) were achieved using all of

these systems. Additionally, DNA addressing APEX chips in distilled water is possible

because the DNA, which is itself a polyanion, is the electrolyte present in the bulk solution

that provides the conductance.

Relationship of Electrophoretic Transport Rate and the Cation/Anion Species

[0038] In addition to the fact that the mobility of the charged analyte species (DNA,

proteins, etc.) is related to the ionic strength of the electrolyte solution, the mobility is also

greatly influenced by the nature of the cationic and anionic species in the electrolyte

solution (see pp 89 of "Capillary Electrophoresis: Principles and Practice" reference).

This particular point is demonstrated for DNA transport in the above-referenced

Biopolymers, Vol. 2, pp. 231-236, 1964 reference. Figure 1 on page 232 of this reference

shows the change in DNA mobility when using electrolytes with different univalent

anions ($Li^+ > Na^+ > K^+ > TMA^+$) at the same ionic strength. Basically, different cations

can have different association constants with the DNA phosphate groups, and/or change

the hydration spheres around the DNA molecules, which leads to a change in their

transport rate.

[0039]   The instant invention also relates to our discoveries concerning the various

parameters, electrolytes (buffers), and other conditions that improve or optimize the speed

of DNA transport, the efficiency of DNA hybridization reactions, and the overall

hybridization specificity in electric field molecular biology devices, especially APEX

microelectronic chips and devices. In particular, this invention relates to our discovery

that low conductance zwitterionic buffer solutions containing a reducing agent provides

good conditions for both rapid electrophoretic DNA transport and efficient hybridization

reactions.

[0040]   The zwitterionic buffer component can be any molecule that can have a

zwitterionic species (no net charge at its pI). In one embodiment, the zwitterionic species

is an amino acid. The amino acid can include, but is not limited to, histidine, alanine,

arginine, glutamic acid, lysine, γ-aminobutyric acid (GABA), etc. In a preferred

embodiment, the amino acid is Histidine. The concentration of histidine can range from

10-200 mM, alternatively 10-100 mM, alternatively 50-100 mM, alternatively about 50

mM, alternatively about 100 mM, at or near the pI (isoelectric point ~7.47). The concentration of histidine in the buffer will depend on the salt composition and can be determined by one of ordinary skill in the art.

[0041]   The advantages of the histidine buffer is particularly important for the APEX chip type devices. These particular devices (as opposed to the micromachined type devices) have limitations as to the amount of current and voltages that can be applied. This limitation makes it difficult to achieve both rapid transport and efficient hybridization using the same buffer system.

[0042]   In one embodiment, DNA transport was carried out in a low conductance buffer (cysteine or alanine) where the limited current/voltage still produced rapid transport. Under these conditions, the DNA accumulated at the test site, but did not hybridize as efficiently. After transport in these low conductance buffers, the solution was changed to a high salt buffer (> 100 mM sodium chloride or sodium phosphate), which then produced an efficient hybridization at the test site.

[0043]   Table 2 shows the results for a series of experiments that correlate the parameters of buffer capacity, pH, and the conductivity, with DNA accumulation and hybridization sensitivity (efficiency) using the APEX chip device.

TABLE 2

Solution	Buffer Capacity pH 4-10		pH at pI	Conductivity (μS)	Relative DNA Transport Rate	SA-Biotin T12 Sensitivity	Hybridization Sensitivity of DNA
β-Alanine	pK$_1$ - 3.6 pK$_2$ - 10.2	+	7.3	10.0	++++ (fastest)	3×10^6	
Taurine	pK$_1$ - 1.5 pK$_2$ - 8.7	+/-	4.6	4.5	++++	$> 7.5 \times 10^{10}$	
Cysteine	pK$_1$ - 1.7 pK$_2$ - 8.3 pK$_3$ - 10.8	+/-	5.2	25.0	++++	3×10^7	7.5×10^{10}
Histidine	pK$_1$ - 1.8 pK$_2$ - 6.0 pK$_3$ - 9.0	+++	7.6	212.0 (172.0 hi purity)	+++	3×10^6	3×10^6
Lysine	pK$_1$ - 2.2 pK$_2$ - 8.9 pK$_3$ - 10.3	++	9.6	477.0	++	$> 7.5 \times 10^{10}$	
NaPO$_4$	Complex	+	7.4[1]	1,400.0	+ (slowest)		

[0044] Table 2 clearly shows the correlation of DNA transport (accumulation) with low conductivity (β-alanine, taurine, cysteine, histidine). The table also shows good sensitivity for the streptavidin/biotin probe affinity reaction using β-alanine, cysteine, and histidine. As reflected in the sensitivity data in Table 2, histidine provides over four orders of magnitude better hybridization efficiency then either cysteine or other buffers, such as 20 mM NaPO$_4$. The improvement relative to cysteine is at least a factor of 10, alternatively a factor of 10^2, and alternatively at least a factor of 10^4. Histidine was found to provide both good transport and good DNA/DNA hybridization efficiency.

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[1]   20mM NaPO$_4$ adjusted to pH 7.4.

Histidine Mechanism of Action

[0045]   During the transport and addressing procedures for DNA concentration and hybridization, the pH immediately above the positively biased electrode is found to be lowered, in a buffer dependent fashion. In separate experiments, it was observed for passive hybridization at acidic pH that histidine can facilitate hybridization when possessing a net positive charge but not when neutral. The ability of these histidine and associated buffers to facilitate electronic hybridization is linked to four important properties: (1) the ability to maintain target DNA in a relatively denatured state, (2) the ability to facilitate electric field concentration of DNA, (3) the ability to buffer acidic conditions present at the positively biased microlocation, (4) the ability to acquire a net positive charge capable of shielding or diminishing repulsion between the DNA phosphodiester backbone stabilizing the double-stranded structure. FIG. 3 shows the possible mechanism for histidine stabilization of DNA structures.

[0046]   Basically, as the histidine molecule becomes protonated and more dicationic with a positive charge on both the α-amino group and imidazole ring, the molecule begins to stabilize the double-stranded DNA structures, promoting hybridization at the positive electrode on the APEX chip. Cations, dications, and polycations are known to help stabilize DNA/DNA hybrids by reducing the repulsion of the negatively charged phosphate backbones on the double-stranded DNA structure. Indeed, upon examining CPK space filling molecular structures of histidine and ds-DNA, the dicationic histidine species, appears to "fit" well to the phosphate oxygen anion spacing along the DNA backbone. Furthermore, examination of CPK space filling structures suggests that di-histidine and other di-, tri-, and polypeptide structures will further significantly stabilize ds-DNA structures. It is believed that in addition to these peptide structures, a large

number of peptide derivatives and synthetic structures can be designed to stabilize ds-

DNA. It is also possible that the DNA/DNA/Histidine may also form some type of

stabilizing adduct from other electrochemical products being produced at the positive

electrode (hydrogen peroxide, etc.).

[0047] The advantage of the histidine and associated buffers, is particularly

important for the APEX microchip type devices. These particular devices are covered

with thinner permeation layer (about 1 to 10 microns), as opposed to deep well devices

(about 10 to 100 micron permeation layers) and micromachined or macroscopic type

devices (sample preparation, complexity reduction, amplification, electronic dot blots,

etc.), and are generally used at a lower range of currents (about 10 nA to about 5 uA)

and voltages (about 1.2 to about 5 volts). This lower current and voltage reduces

transport rate and hybridization efficiency in the higher conductance buffers and

electrolytes. Generally, in these cases, DNA transport would be carried out in a low

conductance buffer (such as cysteine or alanine) where relatively lower current and

voltage still produces rapid DNA transport. Under these conditions, DNA is rapidly

accumulated at the test site, but does not hybridize efficiently. After transport in these

low conductance buffers, the solution is usually changed to a high salt buffer (>100

mM sodium chloride or sodium phosphate), which then promotes very efficient

hybridization of the concentrated target DNA to the DNA probes at a microlocation test

site.

[0048] While this embodiment utilizes naturally occurring amino acids, such as

histidine, this invention is fully applicable to other natural or synthetic compounds that

have good buffering capacity, low conductivity (or zwitterionic characteristics) and have

properties that allow DNA hybridization to be stabilized by charge stabilization or adduct formation.

Reducing Agents

[0049] The addition of a reducing agent to the zwitterionic buffer system also improves DNA transport to the electrodes. The oxidation products at the electrodes, consisting mainly of $O_2(g)$, free radicals, and peroxide species, have been found to contribute to fluorophore, DNA, and permeation layer damage. In addition, oxidation products of buffer components may also deposit on or into the permeation layer and increase the background signal level of the permeation layer above the electrodes. The presence of a reducing agent significantly reduces water oxidation, water oxidation products, and buffer oxidation products at the positive electrode. In particular, the reduction in the generation of $O_2(g)$ bubbles significantly reduces the amount of turbulence in the area immediately surrounding the electrodes, thereby increasing the rate of accumulation of charged particles (e.g., DNA or RNA) to the microlocations. Additionally or alternatively, the presence of the reducing agent allows the application of a higher current to the electrodes, resulting in improved transportation and/or hybridization.

[0050] The reducing agent can be, but is not limited to, cysteine, dithitreitol (DTT), β-mercaptoethanol, α-thioglycerol, other thiol-containing reducing agents, and combinations thereof. The addition of a reducing agent to the low conductance zwitterionic buffers provides the additional advantages of providing a low fluorescent signal background (~75%), protective effects for DNA, RNA, etc., and faster transport of charged materials. The concentration of reducing agent in the buffer is about 25-700 mM, alternatively about

25-500 mM, alternatively about 25 mM, alternatively about 50 mM, alternatively about

100mM, alternatively about 125 mM, alternatively about 150 mM, alternatively about 250

mM, alternatively about 500 mM. The concentration of reducing agent may depend on

the salt composition and can be determined by one of ordinary skill in the art.

[0051]   In one embodiment, the reducing agent has a thiol group. In this case, the

reducing agent has no charge at a pH below the pI of the thiol group. Therefore, there is

no significant increase (minimal increase) in the conductivity of the buffer solution.

[0052]   In a preferred embodiment, the reducing agent is α-thioglycerol. Although all

of the compounds mentioned above are effective reducing agents, the long carbon chain of

α-thioglycerol (for instance, as compared to β-mercaptoethanol) results in lower solution

conductivity, lower transference number, and less odor. L-cysteine tends to have a lower

rate of DNA binding and a residual background increase. DTT is very expensive

compared to α-thioglycerol. β-Mercaptoethanol is toxic and has a distinct odor. α-

thioglycerol is nontoxic and has very little to no odor.

[0053]   When used in combination with at least one zwitterionic species (e.g., histidine)

at various concentrations, reducing agents, and α-thioglycerol (seen below) in particular,

were found to prevent extensive bubbling on electrode devices.

$$CH_2-CH-CH_2-SH$$
$$\ |\quad\ \ |$$
$$OH\quad OH$$
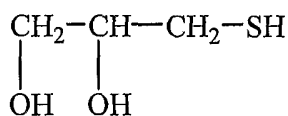
α-thioglycerol

In particular, for APEX devices, the addition of α-thioglycerol was found to prevent

bubbling on Pt chip electrodes at currents in excess of 1 μA. FIG. 4 is a graph of

oxidation/reduction potential sweeps. The oxidation potential of α-thioglycerol in a

solution of 100 mM histidine is less than that necessary for $O_2(g)$ generation from the

electrolysis of water in 100 mM histidine. In addition, the potential at which $O_2(g)$ is

generated is raised significantly (0.25 Volts) in the presence of α-thioglycerol. Therefore,

in the presence of α-thioglycerol, there is sufficient current generated to induce mobility of

a charged biomolecule (e.g., DNA or RNA), but $O_2(g)$ generation is substantially reduced

in favor of the oxidation of α-thioglycerol.

[0054] Additionally, the oxidative products of the reducing agents are less harmful to

the electrode devices. The products of water and α-thioglycerol oxidation are depicted in

FIG. 5. The generation of hydrogen ions from the oxidation of α-thioglycerol helps

consume oxygen and its radicals, reduces the oxidation and formation of polyhistidine

adducts, provides hydrogen ions as charge carriers for electrophoresis and reduces the

local pH to facilitate hybridization. The oxidation products of α-thioglycerol are water

soluble and do not leave a precipitate. For APEX devices, which have a permeation layer

coupled to the electrode, such a precipitate is harmful to the permeation layer. FIG. 6 is an

electrospray mass spectrum of an electrolyzed histidine buffer solution. The peak at m/z

154.1 corresponds to Histidine (His). The peaks at m/z 309.3 and 463.7 correspond to His

adducts, His-His and His-His-His, respectively. FIG. 7 is an electrospray mass spectrum

of an electrolyzed histidine buffer solution containing α-thioglycerol. As apparent in a

comparison of FIGS. 6 and 7, the His adducts at m/z 309 and 463 are significantly reduced

in the presence of α-thioglycerol and peaks corresponding to α-thioglycerol and oxidized

adducts of α-thioglycerol are now present. Additionally, a comparison of the mass spectra

of the histidine/α-thioglycerol buffer (not electrolyzed, see FIG. 8) to the spectrum of the

electrolyzed buffer (see FIG. 7) shows that the buffer components did not change

significantly after electrolysis.

[0055] Transport of DNA is significantly faster in the presence of reducing agents as a result of the higher currents that can be applied when a buffer containing a reducing agent is used. FIG. 9 is a graph of the accumulation of signal, which corresponds to the amount of DNA transported to a particular microlocation after applying a 400 µA pulse for 1 minute. DNA transport appears to be the faster in α-thioglycerol, as compared to DTT and β-mercaptoethanol. The amount of DNA transported in 250 mM DTT is almost twice as much as compared to 50 mM histidine. The amount of DNA transported in 250 mM β-mercaptoethanol is about four-times as much as compared to 50 mM histidine. And the amount of DNA transported in 250 mM α-thioglycerol is over four-times as much as compared to 50 mM histidine.

[0056] In addition, α-thioglycerol does not significantly increase the conductivity of histidine buffers. At working concentrations, α-thioglycerol contributes < 35% to the conductivity of Histidine buffer solutions (see Table 3).

TABLE 3

Buffer	Conductivity (µS/cm)	Std. Dev.
Water	0	0
100 mM Histidine	114	9
50 mM Histidine	56	3
100 mM Histidine/250 mM α-thioglycerol	158	1
50 mM Histidine/125 mM α-thioglycerol	83	2
Conductivity Contribution at 250 mM α-thioglycerol	44 (28%)	
Conductivity Contribution at 125 mM α-thioglycerol	27 (33%)	

[0057] The addition of a reducing agent results in a lower background signal as compared with a buffer without the reducing agent. Table 4 reports the signals where

target oligos P1, P2, P3, FA, and FB were sequentially biased to specific sites on the
chip and allowed to hybridize to capture oligos FA, RB, RA, and IC (internal control).
As seen for Target FA, hybridization only occurred with capture oligo FA.
Additionally, the background signal for all of the other target oligos are lower in the
buffer with α-thioglycerol as compared to the buffer without α-thioglycerol.

TABLE 4

Target	Capture							
	Buffer w/ α-thioglycerol				Buffer w/out α-thioglycerol			
	FA	RB	RA	IC	FA	RB	RA	IC
2 µl P1	17	1	21	1	5	1	6	1
4 µl P1	38	2	37	2	7	1	10	1
8 µl P1	47	2	47	2	9	1	9	1
2 µl P2	25	10	23	10	8	3	6	2
4 µl P2	26	14	26	14	7	3	4	2
8 µl P2	28	18	25	17	6	3	4	2
2 µl P3	27	47	27	40	8	12	7	8
4 µl P3	29	59	31	57	15	14	10	9
8 µl P3	40	83	40	78	16	16	11	13
2 µl FA	534	1	25	1	486	1	21	1
4 µl FA	577	1	31	1	560	1	11	1
8 µl FA	569	1	39	1	658	1	12	1
2 µl FB	77	10	20	7	81	4	12	3
4 µl FB	30	13	28	13	18	4	14	4
8 µl FB	32	17	34	17	19	4	23	6
2 µl RA	15	6	502	5	5	2	534	2
4 µl RA	19	6	642	6	12	4	684	2
8 µl RA	25	8	747	8	7	3	632	3

Heat Denaturation

[0058] Experiments also suggest that the reducing agent, in this case α-thioglycerol,
may act as a chaotropic agent. As seen in Table 5, there is no significant change in
signal intensity when target samples P1, P3, INF B, INF A, and RSV A were addressed
in the zwitterionic buffer containing α-thioglycerol with and without the heat

denaturation step. Therefore, it appears that the target samples that were not subjected

to heat denaturation were denatured by the buffer containing the reducing agent.

TABLE 5

Target	Heat Denaturation	Cartridge 1	Cartridge 2
P1	yes	251	277
P1	no	183	211
P3	yes	2453	2014
P3	no	1915	1974
INF B	yes	563	405
INF B	no	483	272
INF A	yes	852	750
INF A	no	546	464
RSV A	yes	759	994
RSV A	no	931	911

[0059]   Although the foregoing invention has been described in some detail by way

of illustration and example for purposes of clarity and understanding, it will be readily

apparent to those of ordinary skill in the art in light of the teachings of this invention

that certain changes and modifications may be made thereto without departing from the

spirit or scope of the appended claims.

What is claimed is:

1.   A method for the transport of a nucleic acid to an electrode site comprising the steps of

        providing a low conductivity, zwitterionic buffer with a reducing agent to the active electronic system;

        applying current and voltage to an electrode to effect electrophoretic transportation of the nucleic acid towards the electrode.

2.   The method of claim 1, further comprising the step of hybridizing the nucleic acid with a probe located at the electrode, whereby the local pH above the electrode is below the pH of the buffer at its isoelectric point.

3.   The method of claim 1, wherein the reducing agent is α-thioglycerol.

4.   The method of claim 1, wherein the reducing agent is dithiotreitol.

5.   The method of claim 1, wherein the reducing agent is β-mercaptoethanol.

6.   The method of claim 1, wherein a concentration of the reducing agent is about 25-500 mM.

7.   The method of claim 1, wherein a concentration of the reducing agent is about 25 mM.

8.   The method of claim 1, wherein a concentration of the reducing agent is about 125 mM.

9.   The method of claim 1, wherein a concentration of the reducing agent is about 150 mM.

10.   The method of claim 1, wherein a concentration of the reducing agent is about 250 mM.

11.   The method of claim 1, wherein a concentration of the reducing agent is about 500 mM.

12.   The method of claim 1, wherein the low conductivity, zwitterionic buffer is histidine.

13.   The method of claim 12, wherein the low conductivity, zwitterionic buffer further comprises an additional zwitterion.

14.   The method of claim 1, wherein the low conductivity, zwitterionic buffer contains L-histidine.

15.   The method of claim 1, wherein the low conductivity, zwitterionic buffer contains D-histidine.

16.   The method of claim 1, wherein the low conductivity, zwitterionic buffer contains cysteine.

17.   The method of claim 1, wherein the low conductivity, zwitterionic buffer contains alanine.

18.   The method of claim 1, wherein the low conductivity, zwitterionic buffer contains lysine.

19.   The method of claim 1, wherein the low conductivity, zwitterionic buffer contains glutamic acid.

20.   The method of claim 1, wherein the low conductivity, zwitterionic buffer contains γ-aminobutyric acid.

21.   A method for the effective transport and hybridization of a nucleic acid
on an electrode device, comprising the steps of:

        providing a first buffer containing a reducing agent to the electrode

device, wherein the device comprises a plurality of locations having a nucleic

acid probe coupled to a location;

        providing the nucleic acid to the device in the first buffer;

        applying current and potential to the location;

        transporting the nucleic acid to the location;

        changing the buffer to a low conductivity, zwitterionic second buffer;

and

        hybridizing the nucleic acid to the nucleic acid probe at the location.

22.   The method of claim 21, wherein the reducing agent is α-thioglycerol.

23.   The method of claim 21, wherein the reducing agent is dithiotreitol.

24.   The method of claim 21, wherein the reducing agent is β-
mercaptoethanol.

25.   The method of claim 21, wherein a concentration of the reducing agent
is about 25-500 mM.

26.   The method of claim 21, wherein a concentration of the reducing agent
is about 125 mM.

27.   The method of claim 21, wherein a concentration of the reducing agent
is about 25 mM.

28.   The method of claim 21, wherein a concentration of the reducing agent
is about 150 mM.

29.   The method of claim 21, wherein a concentration of the reducing agent is about 250 mM.

30.   The method of claim 21, wherein a concentration of the reducing agent is about 500 mM.

31.   The method of claim 21, wherein the low conductivity, zwitterionic buffer contains histidine.

32.   The method of claim 31, wherein the low conductivity, zwitterionic buffer further comprises an additional zwitterion.

33.   The method of claim 21, wherein the low conductivity, zwitterionic buffer contains cysteine.

34.   The method of claim 21, wherein the low conductivity, zwitterionic buffer contains alanine.

35.   The method of claim 21, wherein the low conductivity, zwitterionic buffer contains lysine.

36.   The method of claim 21, wherein the low conductivity, zwitterionic buffer contains glutamic acid.

37.   The method of claim 21, wherein the low conductivity, zwitterionic buffer contains γ-aminobutyric acid.

38.   A method for the transport and hybridization of nucleic acid on a electronic hybridization device, comprising the steps of:

applying a low conductivity, zwitterionic buffer containing a reducing agent to the electronic hybridization device, wherein the device comprises a location having a nucleic acid probe;

providing a target nucleic acid to the device;

concentrating the target nucleic acid to the location by applying current and potential to the device; and

hybridizing the target nucleic acid with the nucleic acid probe.

39.   The method of claim 38, wherein the reducing agent is α-thioglycerol.

40.   The method of claim 38, wherein the reducing agent is dithiotreitol.

41.   The method of claim 38, wherein the reducing agent is β-mercaptoethanol.

42.   The method of claim 38, wherein a concentration of the reducing agent is about 25-500 mM.

43.   The method of claim 38, wherein a concentration of the reducing agent is about 25 mM.

44.   The method of claim 38, wherein a concentration of the reducing agent is about 125 mM.

45.   The method of claim 38, wherein a concentration of the reducing agent is about 150 mM.

46.   The method of claim 38, wherein a concentration of the reducing agent is about 250 mM.

47.   The method of claim 38, wherein a concentration of the reducing agent is about 500 mM.

48.   The method of claim 38, wherein the low conductivity, zwitterionic buffer contains histidine.

49.   The method of claim 48, wherein the low conductivity, zwitterionic buffer further comprises an additional zwitterion.

50.   The method of claim 38, wherein the low conductivity, zwitterionic buffer contains cysteine.

51.   The method of claim 38, wherein the low conductivity, zwitterionic buffer contains alanine.

52.   The method of claim 38, wherein the low conductivity, zwitterionic buffer contains lysine.

53.   The method of claim 38, wherein the low conductivity, zwitterionic buffer contains glutamic acid.

54.   The method of claim 38, wherein the low conductivity, zwitterionic buffer contains γ-aminobutyric acid.

55. A method for electronically enhancing hybridization of a DNA analyte to a single stranded capture DNA in an electric field at a positively biased location on an electronic device, comprising the steps of:

applying a buffer containing a reducing agent and low conductance, zwitterionic molecules to the device;

applying current to the location on the electronic device in an amount sufficient to produce an electric field at the location, the location being positively biased relative to the DNA analyte;

transporting the DNA analyte to the location;

hybridizing the DNA analyte to the single stranded capture DNA, wherein the positively charged buffer molecules at the location stabilize hybridization between the DNA analyte and the single stranded capture DNA.

56. The method of claim 55, wherein the reducing agent is α-thioglycerol.

57. The method of claim 55, wherein the reducing agent is dithiotreitol.

58. The method of claim 55, wherein the reducing agent is β-mercaptoethanol.

59. The method of claim 55, wherein the low conductivity, zwitterionic buffer molecule is histidine.

60. The method of claim 59, wherein the low conductivity, zwitterionic buffer further comprises an additional zwitterion.

61. The method of claim 55, wherein the low conductivity, zwitterionic buffer molecule is cysteine.

62. The method of claim 55, wherein the low conductivity, zwitterionic buffer molecule is alanine.

63.   The method of claim 55, wherein the low conductivity, zwitterionic buffer molecule is lysine.

64.   The method of claim 55, wherein the low conductivity, zwitterionic buffer molecule is glutamic acid.

65.   The method of claim 55, wherein the low conductivity, zwitterionic buffer molecule is γ-aminobutyric acid.

66.   A method for the transport of a nucleic acid in an electrode device comprising the steps of

adding a double-stranded nucleic acid to a low conductivity, zwitterionic buffer with a reducing agent, wherein the buffer denatures the double-stranded nucleic acid into a first and second strand;

providing the buffer containing the first and second strands to the electrode device;

providing an electrode of the electrode device having a capture sequence, wherein the capture sequence is complementary to the first strand;

applying current and voltage to the electrode at a temperature of less than about 30°C to effect electrophoretic transportation of the first strand towards the electrode; and

hybridizing the first strand of the double-stranded nucleic acid to the capture sequence at the electrode.

67.   The method of claim 66, wherein the current and voltage is applied to the electrode at a temperature of less than about 25°C.

68.   The method of claim 66, wherein the current and voltage is applied to the electrode at a temperature of less than about 20°C.

69.   The method of claim 66, wherein the reducing agent is α-thioglycerol.

70.   The method of claim 66, wherein the reducing agent is dithiotreitol.

71.   The method of claim 66, wherein the reducing agent is β-mercaptoethanol.

72.   The method of claim 66, wherein a concentration of the reducing agent is about 25-500 mM.

73.   The method of claim 66, wherein a concentration of the reducing agent is about 25 mM.

74.   The method of claim 66, wherein a concentration of the reducing agent is about 125 mM.

75.   The method of claim 66, wherein a concentration of the reducing agent is about 150 mM.

76.   The method of claim 66, wherein a concentration of the reducing agent is about 250 mM.

77.   The method of claim 66, wherein a concentration of the reducing agent is about 500 mM.

78.   The method of claim 66, wherein the low conductivity, zwitterionic buffer is histidine.

79.   The method of claim 78, wherein the low conductivity, zwitterionic buffer further comprises an additional zwitterion.

80.   The method of claim 66, wherein the low conductivity, zwitterionic buffer contains L-histidine.

81.   The method of claim 66, wherein the low conductivity, zwitterionic buffer contains D-histidine.

82.   The method of claim 66, wherein the low conductivity, zwitterionic buffer contains cysteine.

83.   The method of claim 66, wherein the low conductivity, zwitterionic buffer contains alanine.

84.   The method of claim 66, wherein the low conductivity, zwitterionic buffer contains lysine.

85.   The method of claim 66, wherein the low conductivity, zwitterionic buffer contains glutamic acid.

86.   The method of claim 66, wherein the low conductivity, zwitterionic buffer contains γ-aminobutyric acid.

87.   A method for the transport of a nucleic acid to an electrode site comprising the steps of

        providing a low conductivity buffer with a reducing agent to the active electronic system;

        applying current and voltage to an electrode to effect electrophoretic transportation of the nucleic acid towards the electrode.

88.   The method of claim 87, further comprising the step of hybridizing the nucleic acid with a probe located at the electrode, whereby the local pH above the electrode is below the pH of the buffer at its isoelectric point.

89.   The method of claim 87, wherein the reducing agent is α-thioglycerol.

90.   The method of claim 87, wherein the reducing agent is dithiotreitol.

91.   The method of claim 87, wherein the reducing agent is β-mercaptoethanol.

92.   The method of claim 87, wherein a concentration of the reducing agent is about 25-500 mM.

93.   The method of claim 87, wherein a concentration of the reducing agent is about 25 mM.

94.   The method of claim 87, wherein a concentration of the reducing agent is about 125 mM.

95.   The method of claim 87, wherein a concentration of the reducing agent is about 150 mM.

96.   The method of claim 87, wherein a concentration of the reducing agent is about 250 mM.

97.   The method of claim 87, wherein a concentration of the reducing agent is about 500 mM.

98.   The method of claim 87, wherein the buffer further comprises a zwitterion.

99.   The method of claim 98, wherein the zwitterion is histidine.

100.   The method of claim 99, wherein the low conductivity buffer further comprises an additional zwitterion.

101.   The method of claim 99, wherein the zwitterion is L-histidine.

102.   The method of claim 99, wherein the zwitterion is D-histidine.

103.   The method of claim 99, wherein the zwitterion is cysteine.

104.   The method of claim 99, wherein the zwitterion is alanine.

105.   The method of claim 99, wherein the zwitterion is lysine.

106.   The method of claim 99, wherein the zwitterion is glutamic acid.

107.   The method of claim 99, wherein the zwitterion is γ-aminobutyric acid.
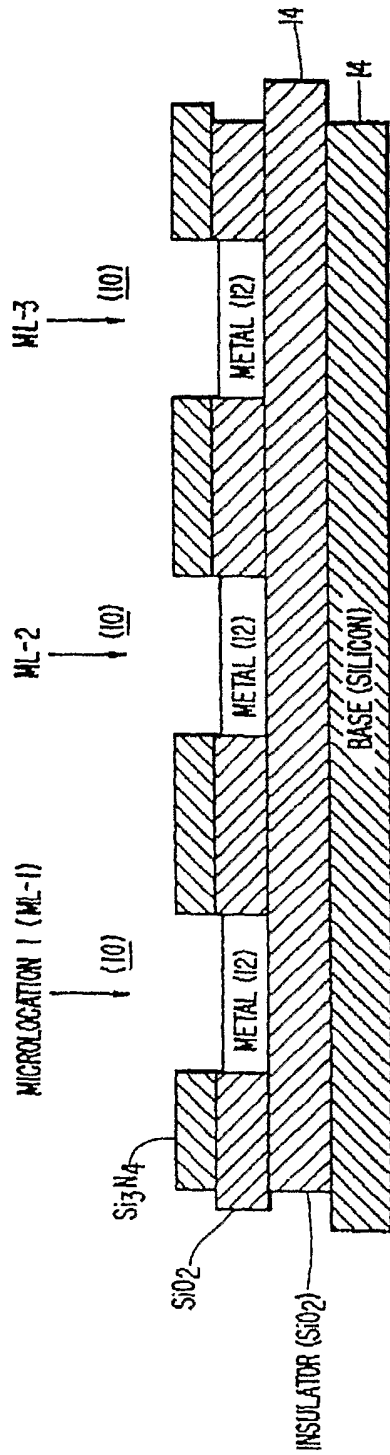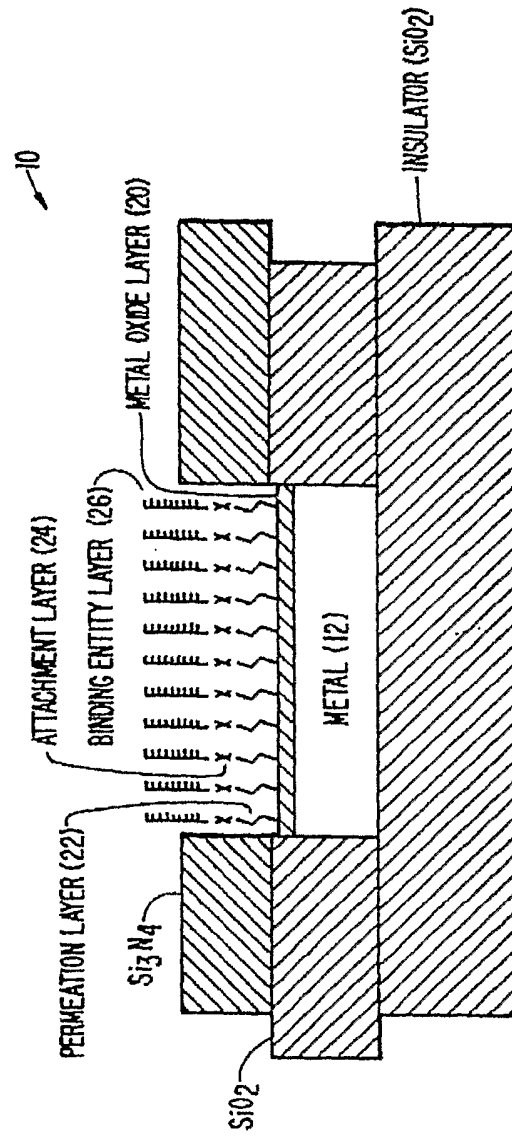
NP1:664196.1

Fig. 1 Self-Addressable Microlocations

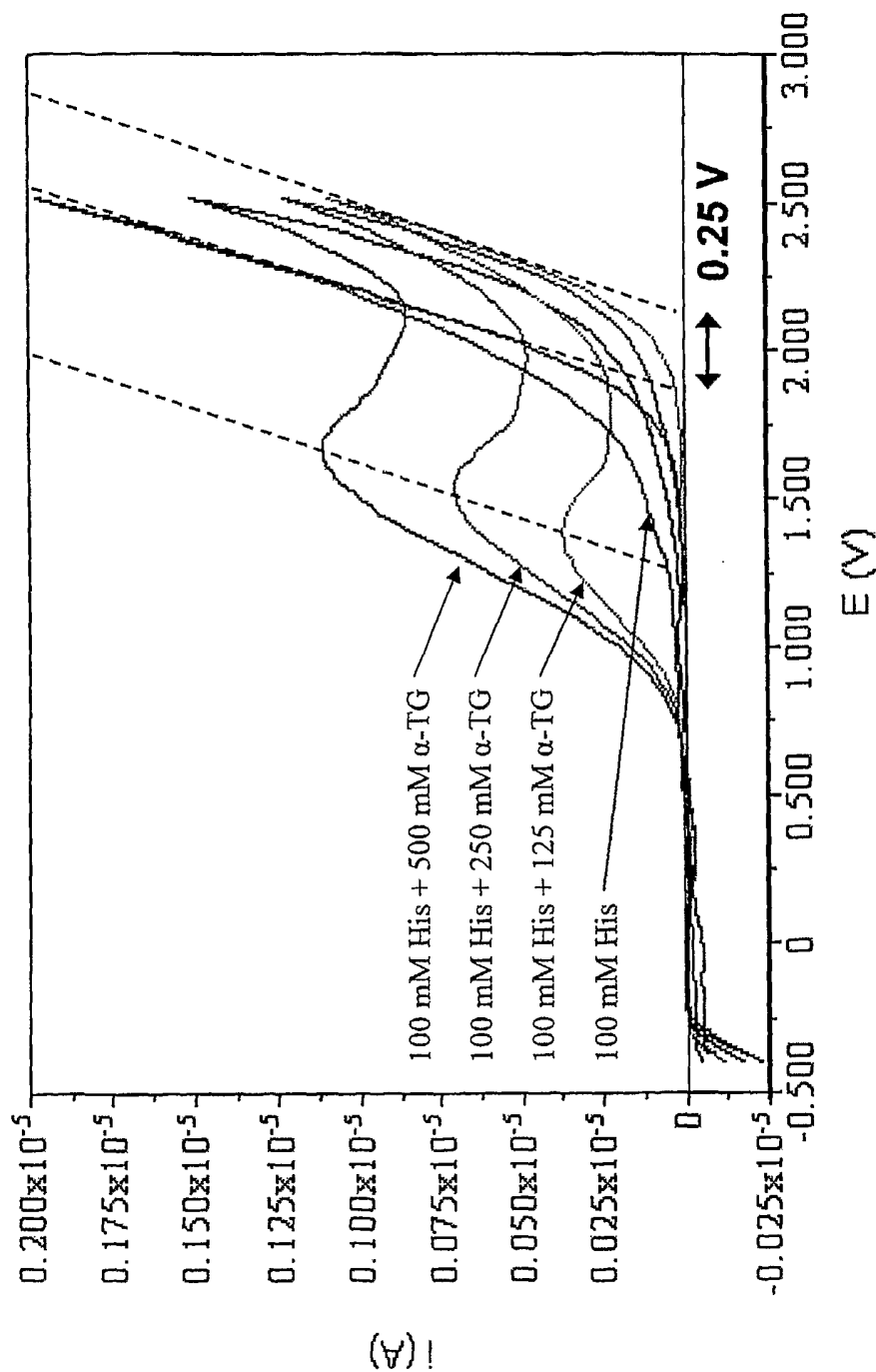

Fig. 2 Cross-section of a Microlocation

Fig. 3 Histidine Stabilization

Fig. 4 Oxidation/Reduction Potential Sweeps

Rxn #1: $2\text{H}_2\text{O} \xrightarrow{\text{Ox}} \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$

Rxn #2: $2\text{R}\text{—SH} \xrightarrow{\text{Ox}} \text{R}\text{—S}\text{—S}\text{—R} + 2\text{H}^+ + 2\text{e}^-$

Rxn #3: $2\text{R}\text{—SH} \xrightarrow{\text{Ox}} 2\text{R}\text{—S}_x\text{O}_y + \text{m}\text{H}^+ + \text{n}\text{e}^-$

Rxn #4: $\text{R}\text{—S}\text{—S}\text{—R} \xrightarrow{\text{Ox}} \text{R}\text{—S}_x\text{O}_y + \text{m}\text{H}^+ + \text{n}\text{e}^-$

Rxn #5: $\xrightarrow{\text{Ox}} \text{R}\text{—OH} + \text{S}_x\text{O}_y + \text{m}\text{H}^+ + \text{n}\text{e}^-$

$$\text{R} \equiv \text{CH}_2\text{-CH-CH}_2\text{-} \atop \qquad\quad |\;\;\;\; | \atop \qquad\;\; \text{OH}\;\; \text{OH}$$
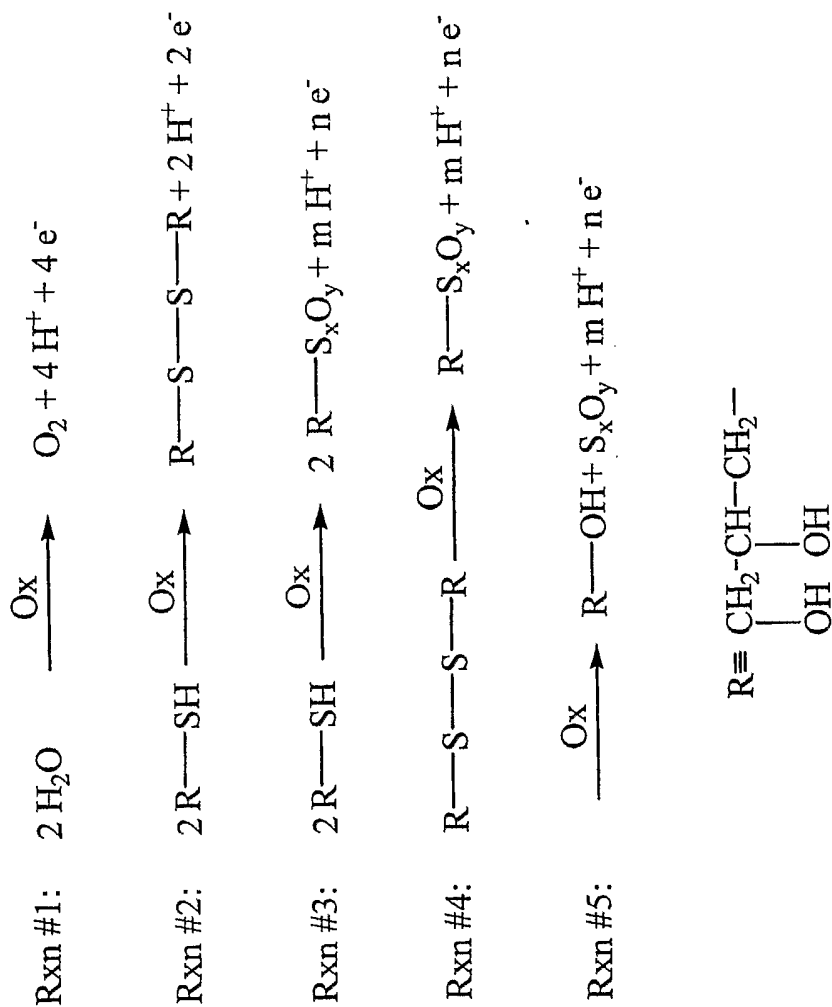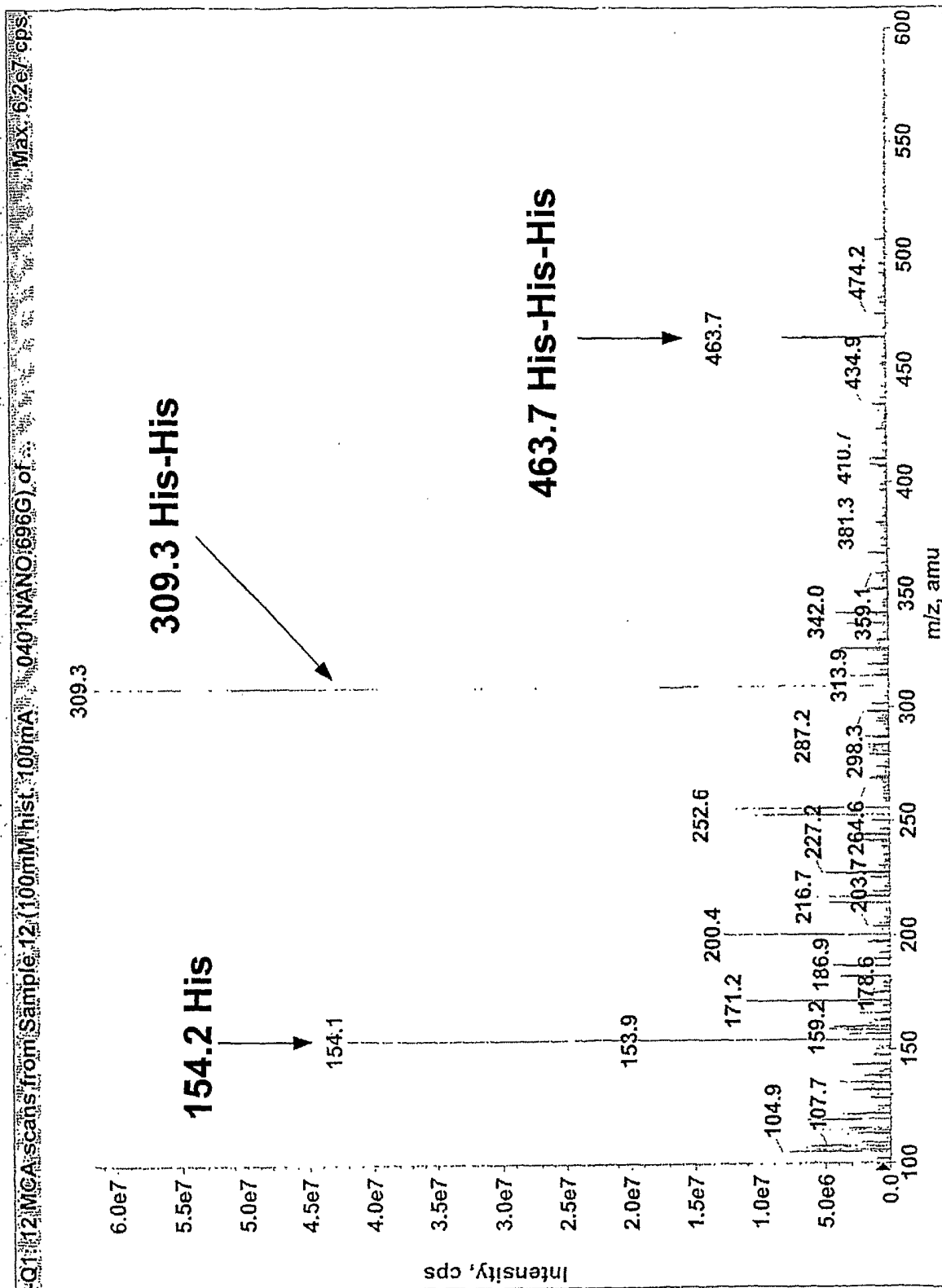
Fig. 5 Products of Water and α-Thioglycerol Oxidation
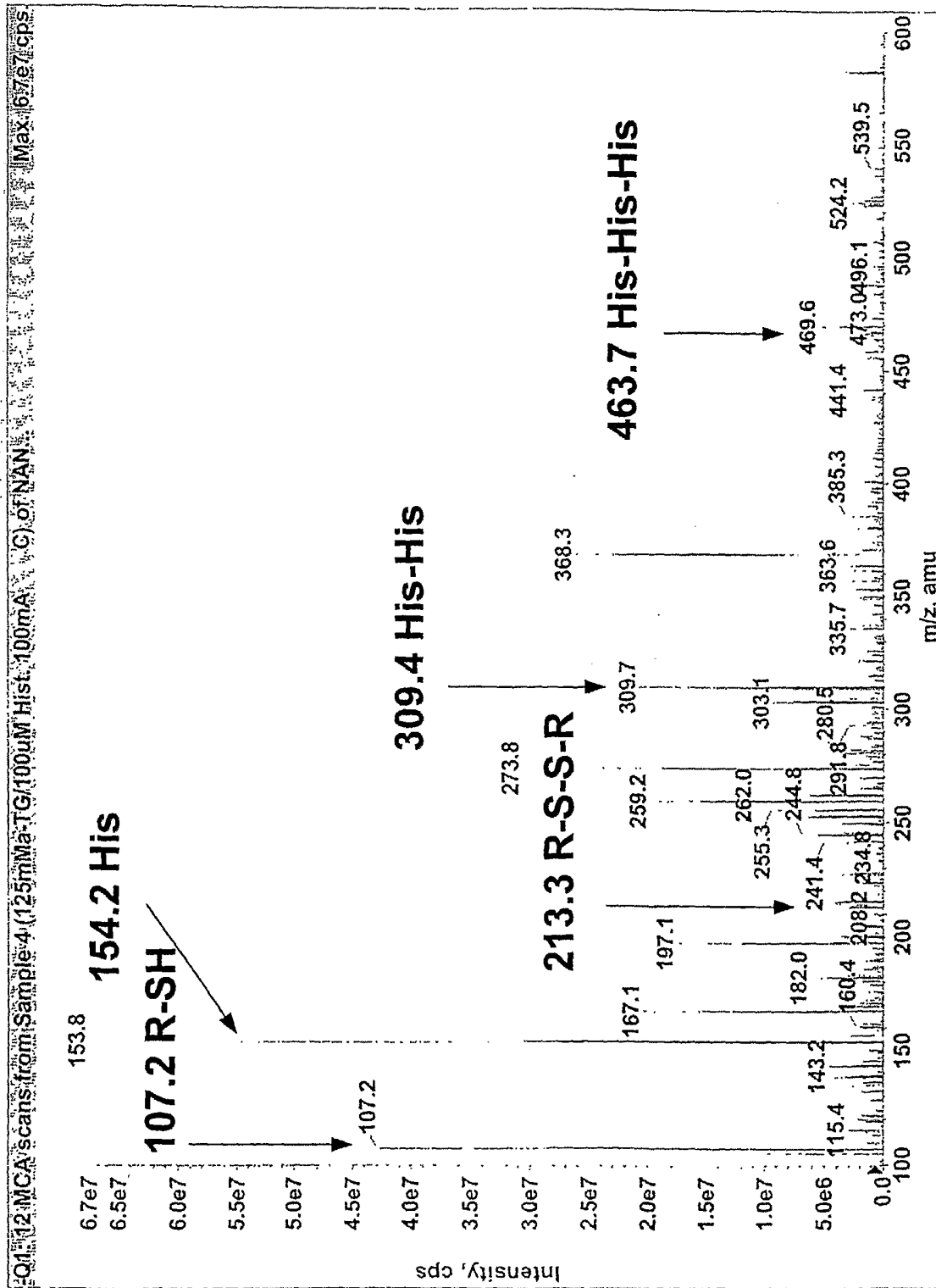
Fig. 6 Mass Spectrum of Electrolyzed Histidine
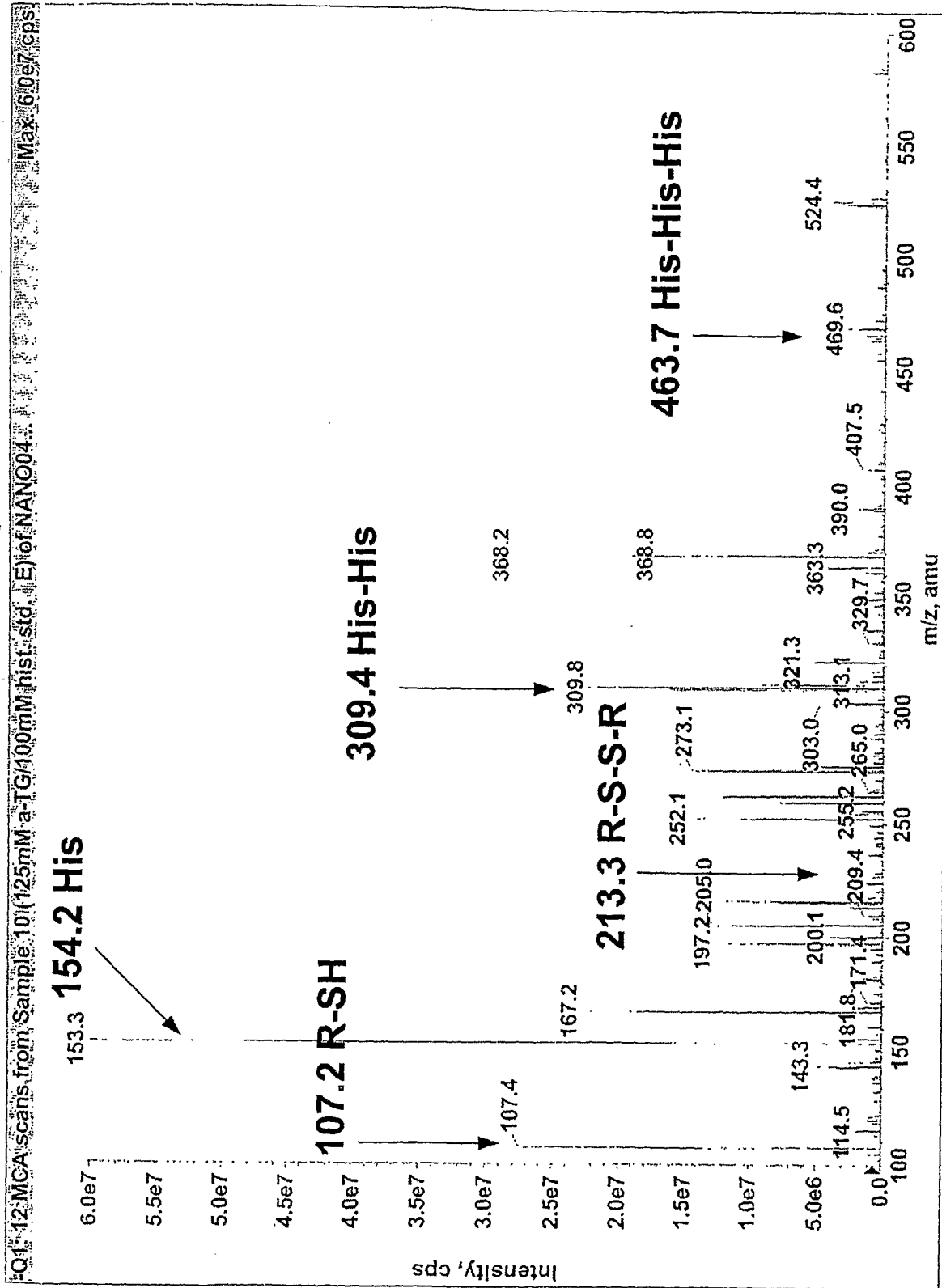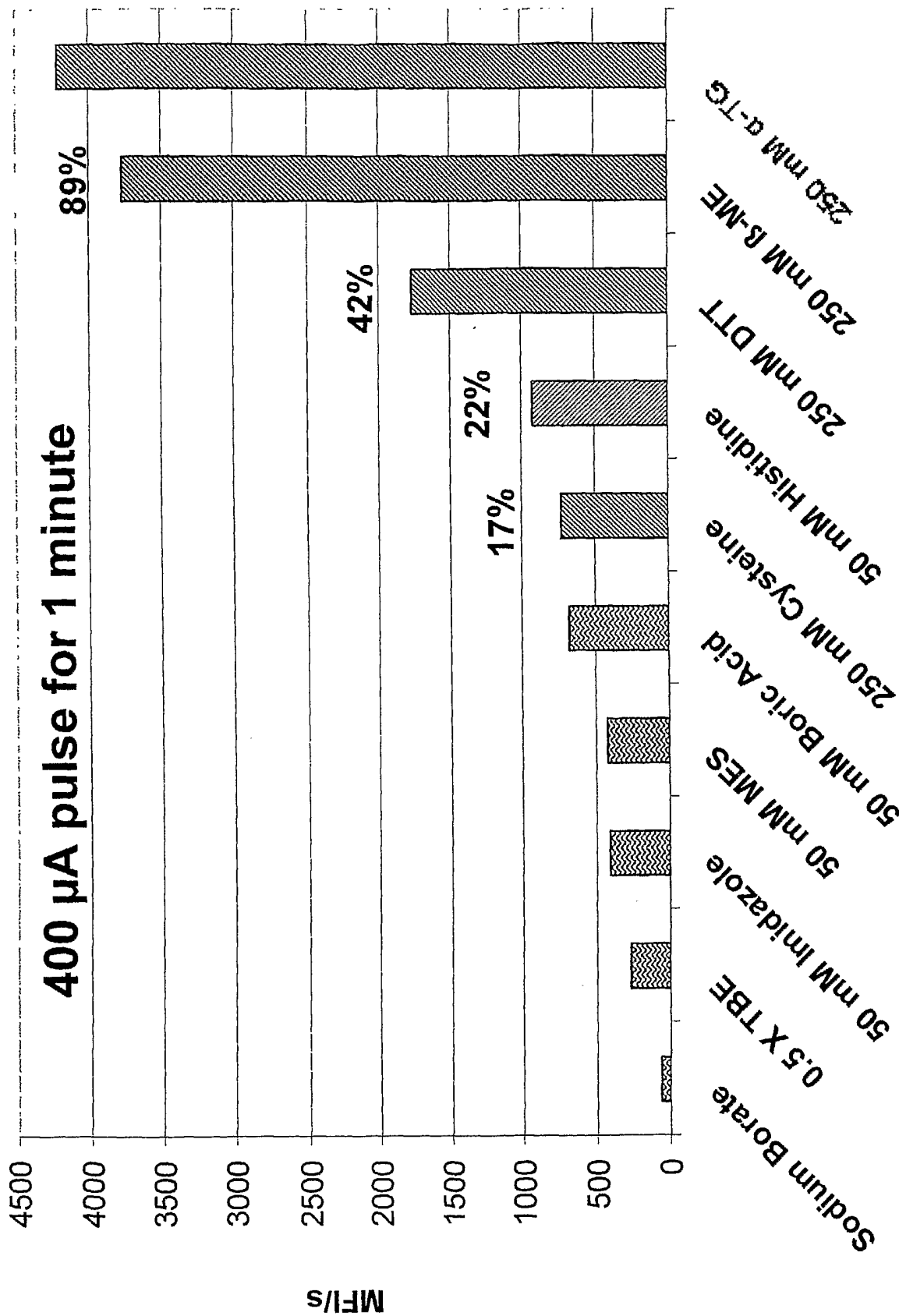
Fig. 7 Mass Spectrum of Electrolyzed α–TG/Histidine

Fig. 8 Mass Spectrum of α–TG/Histidine

Fig. 9 CRD Signal Accumulation