(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 April 2025 (10.04.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/075952 A1

(51) International Patent Classification:
*B05B 5/025* (2006.01) *B05D 1/06* (2006.01)

(21) International Application Number:
PCT/US2024/049386

(22) International Filing Date:
01 October 2024 (01.10.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/587,216 02 October 2023 (02.10.2023) US

(71) Applicants: **THE TRUSTEES OF INDIANA UNIVERSITY** [US/US]; 107 S. Indiana Avenue, Bloomington, Indiana 47405 (US). **WEST VIRGINIA UNIVERSITY BOARD OF GOVERNORS ON BEHALF OF WEST VIRGINIA UNIVERSITY** [US/US]; 886 Chestnut Ridge Road, Morgantown, West Virginia 26506 (US).

(72) Inventors: **CLEMMER, David E.**; 529 South Hawthorne Drive, Bloomington, Indiana 47401 (US). **VALENTINE, Stephen J.**; 769 Augusta Avenue, Morgantown, West Virginia 26501 (US). **LI, Peng**; 886 Chestnut Ridge Road, Morgantown, West Virginia 26506 (US). **JOHNSON, Mathew**; 886 Chestnut Ridge Road, Morgantown, West Virginia 26506 (US). **SHARIF, Daud**; 886 Chestnut Ridge Road, Morgantown, West Virginia 26506 (US). **DEBASTIANI, Anthony**; 886 Chestnut Ridge Road, Morgantown, West Virginia 26506 (US). **DEWSURENDRA, Vikum**; 886 Chestnut Ridge Road, Morgantown, West Virginia 26506 (US).

(74) Agent: **MICHAEL, Jeffrey A.**; BARNES & THORNBURG LLP, 11 South Meridian Street, Indianapolis, Indiana 46204 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG,

**(54) Title:** APPARATUS AND METHOD FOR CONTROLLABLY HEATING NEBULIZED CHARGED PARTICLES

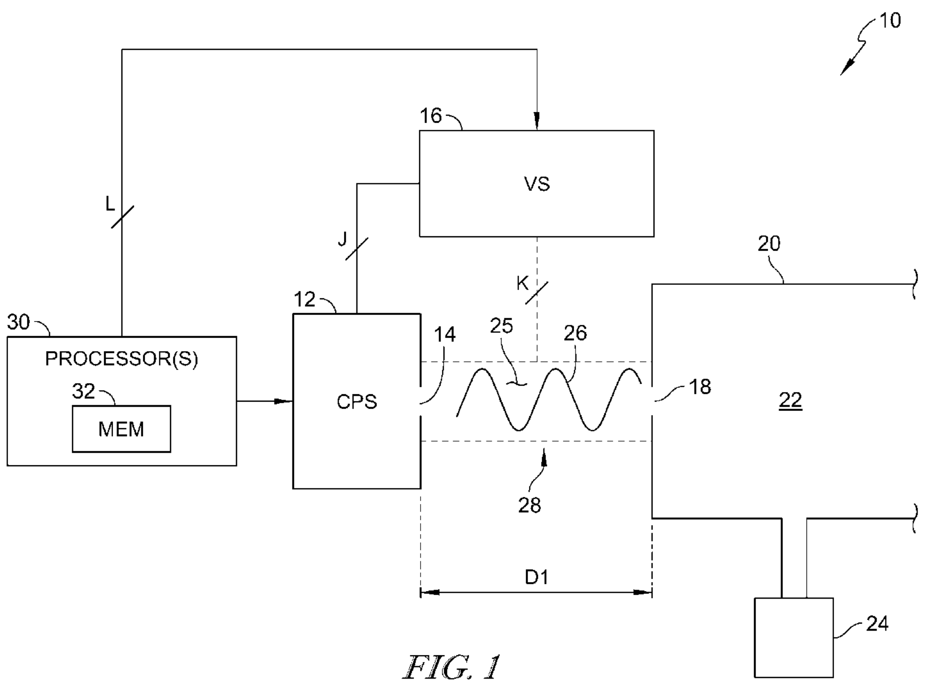


*FIG. 1*

**(57) Abstract:** An apparatus for controllably heating nebulized charged particles may include an electric field region configured to receive the nebulized charged particles therethrough and means for establishing a time-varying electric field within the electric field region such that the nebulized charged particles pass through the time-varying electric field. The time-varying electric field is configured to heat the nebulized charged particles passing therethrough with a target degree of heating.

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KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

**(84) Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**
— *of inventorship (Rule 4.17(iv))*

**Published:**
— *with international search report (Art. 21(3))*

# APPARATUS AND METHOD FOR CONTROLLABLY HEATING NEBULIZED CHARGED PARTICLES

## CROSS REFERENCE TO RELATED APPLICATION

**[0001]** This international patent application claims the benefit of, and priority to, U.S. Provisional Patent Application Ser. No. 63/587,216, filed October 2, 2023, the disclosure of which is expressly incorporated herein by reference in its entirety.

## GOVERNMENT RIGHTS

**[0002]** This invention was made with government support under GM135432 awarded by the National Institute of Health. The United States Government has certain rights in the invention.

## TECHNICAL FIELD

**[0003]** The present disclosure relates generally to processing nebulized charged particles prior to charged particle analysis, and more specifically to processing such nebulized charged particles in a manner which controllably heats the nebulized charged particles prior to, or during, analysis by at least one charged particle analysis instrument.

## BACKGROUND

**[0004]** Generation of charged particles from a sample solution is a conventional charged particle generation technique in which the sample solution is nebulized in a manner which produces charged particle droplets. The charged particle droplets, typically expelled in the form of a plume, may then be fed into a charged particle analysis instrument or system for analysis by at least one charged particle analysis device or instrument.

## SUMMARY

**[0005]** The present invention may comprise one or more of the features recited in the attached claims, and/or one or more of the following features and combinations thereof.

**[0006]** In a first aspect, a method for controllably heating nebulized charged particles may comprise configuring a time-varying electric field to heat the nebulized charged particles passing therethrough with a target degree of heating; and passing the nebulized charged particles through the configured time-varying electric field to heat the nebulized charged particles with the target degree of heating.

**[0007]** A second aspect includes the features of the first aspect, and wherein configuring the time-varying electric field may comprise selecting at least one electrical parameter of the time-varying electric field which will achieve the target degree of heating of the nebulized charged particles.

**[0008]** A third aspect includes the features of the second aspect, and wherein the at least one electrical parameter of the time-varying electric field may comprise one or any combination of frequency, shape, duty cycle, and peak amplitude of the time-varying electric field.

**[0009]** A fourth aspect includes the features of any of the first aspect through the third aspect, and may further comprise establishing the configured time-varying electric field within and along an electric field region.

**[0010]** A fifth aspect includes the features of the fourth aspect, and wherein configuring the time-varying electric field may comprise selecting a length of the electric field region which will achieve the target degree of heating of the nebulized charged particles.

**[0011]** A sixth aspect includes the features of the fourth aspect, and wherein the nebulized charged particles may have a flow rate with which the nebulized charged particles pass through the configured time-varying electric field, and wherein configuring the time-varying electric field may comprise selecting either or both of the at least one electrical parameter and the length of the electric field region which will achieve the target degree of heating of the nebulized charged particles taking into account the flow rate of the nebulized charged particles through the configured time-varying electric field.

**[0012]** A seventh aspect includes the features of any of the first aspect through the sixth aspect, and wherein configuring the time-varying electric field to heat the nebulized charged particles may comprise configuring the time-varying electric field in a manner which controls at least one of peak heating temperature, heating rate, and total heating time of the nebulized charged particles passing through the configured time-varying electric field.

**[0013]** An eighth aspect includes the features of any of the first aspect through the seventh aspect, and wherein the target degree of heating may comprise complete evaporation of the nebulized charged particles exiting the configured time-varying electric field.

**[0014]** A ninth aspect includes the features of any of the first aspect through the seventh aspect, and wherein the target degree of heating may comprise incomplete evaporation of the nebulized charged particles exiting the configured time-varying electric field.

**[0015]** A tenth aspect includes the features of any of the first aspect through the seventh aspect, and wherein the nebulized charged particles may carry at least one protein, and wherein the target degree of heating may comprise at least partial unfolding of the at least one protein.

**[0016]** In an eleventh aspect, a method of analyzing charged particles may comprise generating nebulized charged particles; passing the generated nebulized charged particles through the configured time-varying electric field of any of the first aspect through the tenth aspect to heat the generated nebulized charged particles with the target degree of heating; and analyzing at least one molecular characteristic of the generated nebulized charged particles exiting the configured time-varying electric field.

**[0017]** In a twelfth aspect, a method of analyzing charged particles may comprise generating nebulized charged particles; passing the generated nebulized charged particles into a pressure-controlled charged particle analysis instrument; within the pressure-controlled charged particle analysis instrument, passing the generated nebulized charged particles through the configured time-varying electric field of any of the first aspect through the tenth aspect to heat the generated nebulized charged particles with the target degree of heating; and within the pressure-controlled charged particle analysis instrument, analyzing at least one molecular characteristic of the generated nebulized charged particles exiting the configured time-varying electric field.

**[0018]** In a thirteenth aspect, a method of analyzing charged particles may comprise generating nebulized charged particles; passing the generated nebulized charged particles into a pressure-controlled charged particle analysis instrument; within the pressure-controlled charged particle analysis instrument, analyzing or processing the generated nebulized charged particles according to a first molecular characteristic; within the pressure-controlled charged particle analysis instrument, passing the nebulized charged particles, analyzed or processed according to the first molecular characteristic, through the configured time-varying electric field of any of the first aspect through the tenth aspect to heat the generated nebulized charged particles with the target degree of heating; and within the pressure-controlled charged particle analysis instrument, analyzing the generated nebulized charged particles exiting the configured time-varying electric field according to the first or a second molecular characteristic.

**[0019]** In a fourteenth aspect, an apparatus for controllably heating nebulized charged particles may comprise an electric field region configured to receive the nebulized charged particles therethrough; and means for establishing a time-varying electric field within the electric field region such that the nebulized charged particles pass through the time-varying electric field, the time-varying electric field configured to heat the nebulized charged particles passing therethrough with a target degree of heating.

**[0020]** A fifteenth aspect includes the features of the fourteenth aspect, and wherein the time-varying electric field may be configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring at least one electrical parameter of the time-varying electric field in a manner which will achieve the target degree of heating of the nebulized charged particles, and wherein the at least one electrical parameter may comprise one or any combination of frequency, shape, duty cycle, and peak amplitude of the time-varying electric field.

**[0021]** A sixteenth aspect includes the features of any of the fourteenth aspect through the fifteenth aspect, and wherein the electric field region may have a length in and along which the time-varying electric field is established, and wherein the time-varying electric field may be configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring the length of the electric field region in a manner which will achieve the target degree of heating of the nebulized charged particles.

**[0022]** A seventeenth aspect includes the features of any of the fourteenth aspect through the sixteenth aspect, and wherein the nebulized charged particles may have a flow rate with which the nebulized charged particles pass through the electric field region, and wherein the time-varying electric field may be configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring either or both of the at least one electrical parameter and the length of the electric field region which will achieve the target degree of heating of the nebulized charged particles taking into account the flow rate of the nebulized charged particles through the electric field region.

**[0023]** An eighteenth aspect includes the features of any of the fourteenth aspect through the seventeenth aspect, and wherein the time-varying electric field may be configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring the time-varying electric field in a manner which controls at least one of peak heating temperature, heating rate, and total heating time of the nebulized charged particles passing through the electric field region.

**[0024]** A nineteenth aspect includes the features of any of the fourteenth aspect through the eighteenth aspect, and wherein the time-varying electric field may be configured to heat the nebulized charged particles passing therethrough with the target degree of heating by heating the nebulized charged particles to complete evaporation.

**[0025]** A twentieth aspect includes the features of any of the fourteenth aspect through the eighteenth aspect, and wherein the time-varying electric field may be configured to heat the nebulized charged particles passing therethrough with the target degree of heating by heating the nebulized charged particles in a manner which does not completely evaporate the nebulized charged particles.

**[0026]** A twenty-first aspect includes the features of any of the fourteenth aspect through the eighteenth aspect, and wherein the nebulized charged particles may carry at least one protein, and wherein the time-varying electric field may be configured to heat the nebulized charged particles passing therethrough with the target degree of heating by heating the nebulized charged particles in a manner which results in at least partial unfolding of the at least one protein.

**[0027]** A twenty-second aspect includes the features of any of the fourteenth aspect through the twenty-first aspect, and wherein the means for establishing the time-varying electric field within the electric field region may further comprise means for generating the nebulized charged particles.

**[0028]** A twenty-third aspect includes the features of the twenty-second aspect, and wherein the means for establishing the time-varying electric field within the electric field region may comprise: a rigid substrate; a capillary having one end fluidly coupled to a sample solution and an opposite end forming an emitter with an emitter tip defining an orifice therein, a portion of the emitter being affixed to the rigid substrate; a transducer coupled to the rigid substrate; a waveform generator electrically coupled to the transducer and configured to produce time-varying waveforms, the transducer responsive to the time-varying waveforms to vibrate the rigid substrate to cause the emitter tip to oscillate; a pump configured to pump the sample solution through the capillary so as to exit the orifice of the oscillating emitter tip into the electric field region in the form of a plume of the nebulized charged particles; and a voltage source configured to apply a DC voltage to the emitter tip to induce the time-varying electric field in the plume of the nebulized charged particles exiting the oscillating emitter tip.

**[0029]** A twenty-fourth aspect includes the features of any of the fourteenth aspect through the twenty-first aspect, and wherein the means for establishing the time-varying electric field within the electric field region may comprise: first and second conductive sheets or plates spaced apart from one another to define the electric field region therebetween; and at least one voltage source configured to apply a time-varying voltage to and between the first and second sheets or plates to establish the time-varying electric field between the first and second sheets or plates.

**[0030]** A twenty-fifth aspect includes the features of the twenty-fourth aspect, and may further comprise a charged particle analysis instrument, and wherein the electric field region may be external to the charged particle analysis instrument and positioned adjacent to a charged particle inlet of the charged particle analysis instrument such the nebulized charged particles exiting the electric field region enter the charged particle inlet of the charged particle analysis instrument.

**[0031]** A twenty-sixth aspect includes the features of the twenty-fourth aspect, and may further comprise a charged particle analysis instrument, and wherein the electric field region may be positioned within the charged particle analysis instrument.

**[0032]** A twenty-seventh aspect includes the features of any of the twenty-fifth aspect through the twenty-sixth aspect, and may further comprise a charged particle source configured to generate the nebulized charged particles and supply the nebulized charged particles to the electric field region.

**[0033]** These and other features of the present disclosure will become more apparent from the following description of the illustrative embodiments.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0034]** Fig. 1 is a simplified diagram of an embodiment of a system for analyzing nebulized charged particles including an apparatus for controllably heating the nebulized charged particles prior to charged particle analysis;

**[0035]** Fig. 2 is a simplified diagram of an embodiment of the system of Fig. 1 including an embodiment of an apparatus for generating and controllably heating nebulized charged particles prior to charged particle analysis;

**[0036]** Fig. 3 is a simplified diagram of a portion of the apparatus of Fig. 2 illustrating oscillation of a capillary emitter in response to vibrations induced on a capillary by a waveform generator;

**[0037]** Fig. 4 is a plot of electric field vs. time illustrating an example time-varying electric field generated in a region of the nebulized charged particles by the vibrating capillary emitter of the apparatus of Fig. 2;

**[0038]** Fig. 5 is a plot of charge state vs. applied DC voltage for an example solution sample containing Ubiquitin using the system of Fig. 2;

**[0039]** Fig. 6 is a plot of relative abundance vs. mass-to-charge ratio of Ubiquitin using the system of Fig. 2 and operated with an applied DC voltage of 0 volts which corresponds to $V_{DC1}$ in the plot of Fig. 5;

**[0040]** Fig. 7 is a plot of relative abundance vs. mass-to-charge ratio of Ubiquitin using the system of Fig. 2 and operated with an applied DC voltage of 350 volts which corresponds to $V_{DC2}$ in the plot of Fig. 5;

**[0041]** Fig. 8 is a plot of relative abundance vs. mass-to-charge ratio of Ubiquitin using the system of Fig. 2 and operated with an applied DC voltage of 600 volts which corresponds to $V_{DC3}$ in the plot of Fig. 5;

**[0042]** Fig. 9 is a plot of relative abundance vs. mass-to-charge ratio of Ubiquitin using the system of Fig. 2 and operated with an applied DC voltage of 1000 volts which corresponds to $V_{DC4}$ in the plot of Fig. 5;

**[0043]** Fig. 10 is a simplified diagram of another embodiment of the system of Fig. 1 including another embodiment of an apparatus for controllably heating the nebulized charged particles prior to charged particle analysis;

**[0044]** Fig. 11 is a simplified diagram of yet another embodiment of the system of Fig. 1 including the apparatus for controllably heating the nebulized charged particles of Fig. 10 incorporated into a front end of a charged particle analysis instrument; and

**[0045]** Fig. 12 is a simplified diagram of still another embodiment of the system of Fig. 1 including the apparatus for controllably heating the nebulized charged particles of Fig. 10 incorporated into another portion of the charged particle analysis instrument.

## DETAILED DESCRIPTION OF THE DRAWINGS

**[0046]** While the concepts of the present disclosure are susceptible to various modifications and alternative forms, specific exemplary embodiments thereof have been shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that there is no intent to limit the concepts of the present disclosure to the particular forms disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives consistent with the present disclosure and the appended claims.

**[0047]** References in the specification to "one embodiment", "an embodiment", "an example embodiment", etc., indicate that the embodiment described may include a particular feature, structure, or characteristic, but every embodiment may not necessarily include the particular feature, structure, or characteristic. Moreover, such phrases may or may not necessarily refer to the same embodiment. Further, when a particular feature, structure, or characteristic is described in connection with an embodiment, it is submitted that it is within the knowledge of one skilled in the art to effect such feature, structure, or characteristic in connection with other embodiments whether or not explicitly described. Further still, it is contemplated that any single feature, structure, or characteristic disclosed herein may be combined with any one or more other disclosed feature, structure, or characteristic, whether or not explicitly described, and that no limitations on the types and/or number of such combinations should therefore be inferred.

**[0048]** Definitions:

**[0049]** As used herein, the term “capillary vibrating sharp-edge spray ionization” or “CVSSI” means and refers to a conventional field-free (i.e., voltage-free) ionization technique which generates a droplet stream from a liquid sample supplied to a capillary coupled to the sharp edge of a surface that is mechanically vibrated. This technique provides for nebulization of the liquid sample infused directly through the capillary without the use of a nebulization gas or application of an electric field.

**[0050]** The term “field-enabled capillary vibrating sharp-edge spray ionization” or “FE-CVSSI” means and refers to CVSSI in which a DC potential is applied to the capillary. Conventional FE-CVSSI is generally understood to be comparable to conventional electrospray ionization (ESI) in terms of makeup and charge state distributions of the generated analyte ions.

**[0051]** Referring now to Fig. 1, an embodiment is shown of a system 10 for analyzing nebulized charged particles including an apparatus for controllably heating nebulized charged particles prior to charged particle analysis with a time-varying electric field. In the illustrated embodiment, the system 10 includes a charged particle source 12 having a charged particle outlet 14 configured to produce nebulized charged particle droplets from a solution containing a sample, e.g., containing one or more analytes, to be analyzed (hereinafter “sample solution”). The nebulized charged particle droplets are typically, although not exclusively, expelled by the charged particle source 12 from the charged particle outlet 14 in the form of a plume. In the embodiment illustrated in Fig. 1, the sample solution is not shown and will be understood to be included within, or as part of, the charged particle source 12.

**[0052]** In some embodiments, the sample solution may be an aqueous solution containing the sample from which the charged particle droplets are to generated (and subsequently analyzed), although in alternate embodiments the solution may be or include non-aqueous solutions and/or solvents. In still other embodiments, the sample solution may be the sample itself, e.g., blood or other bodily fluid. In some embodiments, the sample solution may be or contain a biological sample containing one or multiple biological components. Alternatively or additionally, the sample solution may be or contain one or more non-biological samples containing one or multiple non-biological components. In any case, the charged particle source 12 may illustratively be conventional, examples of which may include, but are not limited to, one or any combination of an electrospray ionization (ESI) source, a surface acoustic wave nebulization source, a mechanospray ionization source, a CVSSI source, an FE-CVSSI source, and any conventional ultrasonic nebulization source.

**[0053]** In some embodiments, operation of the charged particle source 12 is controlled by a voltage source 16 configured to produce one or more time-varying, i.e., AC, output voltage(s) and, in some embodiments, one or more constant, i.e., DC, output voltage(s). In the embodiment illustrated in Fig. 1, the voltage source 16 is electrically coupled to the charged particle source 12 via a number, J, of signal paths, where J may be any positive integer. The voltage source 16 is illustratively operable to control operation of the charged particle source 12 by producing one or more DC and/or one or more AC voltages on one or more of the J signal paths. The voltage source 16 may, in some embodiments, be a single voltage source, although in alternate embodiments the voltage source 16 may be provided in the form of multiple voltage sources each configured to produce one or more AC voltages and, in some embodiments, one or more DC voltages.

**[0054]** In the illustrated embodiment, the nebulized charged particles generated by the charged particle source 12 are controllably heated by a time-varying electric field, as will be described in detail below, after which the controllably heated charged particles enter a charged particle inlet 18 of a charged particle analysis instrument or system 20. Generally, charged particle inlet 18 defines a charged particle inlet to a region 22 of the charged particle analysis instrument or system 20 which is pumped by a conventional pump 24 to a sub-atmospheric pressure, i.e., a vacuum. In some alternate embodiments, the time-varying electric field may be generated within the charged particle analysis instrument or system 20, i.e., in a time-varying electric field region which is under vacuum, and in such embodiments the nebulized charged particles generated by the charged particle source 12 are fed directly, i.e., without controlled heating, into the charged particle inlet 18 of the charged particle analysis instrument or system 20 where the nebulized charged particles are thereafter controllably heated by the time-varying electric field generated within the charged particle analysis instrument or system 20. Non-limiting examples of two such embodiments are illustrated in Figs. 11 and 12, and will be described in detail below.

**[0055]** In any case, the charged particle analysis instrument or system 20, in embodiments which include it, may be or include one or a combination of any conventional charge particle analysis instrument(s), examples of which may include, but are not limited to, one or any combination of conventional instruments for separating charged particles as a function of one or more molecular characteristic such as, but not limited to, mass-to-charge ratio, e.g., one or more conventional mass spectrometers, mass-to-charge ratio and charge, e.g., one or more conventional charge detection mass spectrometers, mobility, e.g., one or more conventional ion mobility spectrometers, retention time, or the like. Alternatively or additionally, any such charged particle analysis instrument(s), if included, may illustratively include one or more conventional instruments for processing charged particles, examples of which may include, but are not limited to, one or any combination of at least one instrument for collecting or storing charged particles, e.g., one or more conventional ion traps, at least one instrument for guiding or transporting charged particles, e.g., one or more conventional, RF-only multi-pole instruments, at least one instrument for filtering charged particles according to a molecular characteristic, e.g., one or more conventional multi-pole instruments configured to filter charged particles according to a specified range of mass-to-charge ratio, at least one instrument for dissociating ions, e.g., one or more conventional charged particle fragmentation instruments or devices, and at least one instrument for normalizing or shifting ion charge states. In some embodiments, the voltage source 16 may be configured to produce one or more AC voltages and, in some embodiments, one or more DC voltages, for controlling operation of the charged particle analysis instrument or system 20, although in alternate embodiments at least one operating feature of the charged particle analysis instrument or system 20 may be controlled by a separate voltage source configured to produce one or more AC voltages and, in some embodiments, one or more DC voltages (e.g., in the form of a single voltage source configured to produce the voltage(s), or in the form of multiple voltage sources each configured to produce the voltage(s)).

**[0056]** The charged particle outlet 14 of the charged particle source 12 is spaced apart from the charged particle inlet 18 of the charged particle analysis instrument 20 by a distance D1. In the space 25, which may also be referred to as an electric field region 25, defined by D1 between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet 18 of the charged particle analysis instrument 20, a time-varying electric field 26 is generated such that charged particles exiting the charged particle outlet 14 of the charged particle source 12 travel through the time-varying electric field 26 on their way into the charged particle inlet 18 of the charged particle analysis instrument 20. In some embodiments, a time-varying electric field generation device 28 is disposed between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet 18 of the charged particle analysis instrument 20, and is electrically coupled to the voltage source 16 via any number, K, of signal paths, wherein K may be any positive integer, as shown by dashed-line representation in Fig. 1. In embodiments which include it, the time-varying electric field generation device 28 defines the electric field region 25 between the charged particle source 12 and the charged particle analysis instrument 20. The time-varying electric field generation device 28 is responsive to at least one AC voltage or to a combination of at least one AC voltage and at least one DC voltage to generate the time-varying electric field 26 within the electric field region 25. A non-limiting example of the electric field generation device 28 is depicted in Fig. 10, and will be described in detail below.

**[0057]** Operation of the charged particle source 12 and/or operation of the voltage source 16 may illustratively be controlled by at least one conventional processor 30. In embodiments which include the processor(s) 30, one or more memory devices 32 illustratively has/have stored therein instructions which are executable by the processor(s) 30 to cause the processor(s) 30 to control operation of the charged particle source 12 and/or operation of the voltage source 16. In some embodiments, for example, the charged particle source 12 may include a pump operable to draw the sample solution from a container, and in such embodiments the memory device(s) 32 may include instructions executable by the processor(s) 30 to control such a pump, and/or other device associated with the operation of the charged particle source 12, in a conventional manner. In other embodiments which include such pump and/or other device associated with the operation of the charged particle source 12, any such pump and/or other device may be manually controllable or may be configured to be manually programmed for operation. In some embodiments, the memory device(s) 32 may include instructions executable by the processor(s) 30 to control operation of the voltage source 16 to control the frequency, shape, duty cycle, and/or amplitude of one or more AC voltage signals produced thereby and, in some embodiments, to control the amplitude and/or polarity of one or more DC voltage signals produced thereby, and in such embodiments control outputs of the processor(s) 32 is/are electrically connected to one or more control inputs of the voltage source 16 via any number, L, of signal paths, where L may be any positive integer. In other embodiments, the voltage source 16 may be manually controllable or programmable to produce one or more AC voltages with selected frequency, shape, duty cycle, and/or amplitude and, in some embodiments, to produce one or more DC voltages with selected amplitude and/or polarity. In any case, the at least one processor 30 may be any conventional circuit or circuits configured to execute instructions stored in the memory device(s) 32, examples of which may include, but are not limited to, one or more conventional microprocessors, one or more microcontrollers, or the like. The at least one memory device 32 may likewise be any conventional memory circuit or circuits configured to store instructions therein for execution by the processor(s) 30. In some alternate embodiments, the processor(s) 30 and/or the memory device(s) 32 may take the form of analog circuitry designed to carry out the tasks described herein.

**[0058]** As will be described in further detail below, in the embodiment of the system 10 illustrated in Fig. 1, the time-varying electric field 26 established in the electric field region 25, between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet 18 of the charged particle analysis instrument or system 20, is configured in a manner which controllably heats the charged particle droplets generated by the charged particle source 12 prior to entrance into the charged particle analysis instrument or system 20 via the charged particle inlet 18. In some alternate embodiments, some of which are depicted by example in Figs. 11 and 12, the time-varying electric field may be established within the charged particle analysis instrument or system 20, and in such embodiments will operate to controllably heat charged particle droplets within the charged particle analysis instrument or system 20.

**[0059]** As used herein, the term “controllably heat,” means to control a degree, i.e., amount, level, or extent, of heating of nebulized charged particles, i.e., charged particle droplets, in terms of one or more heating parameters including, for example, but not limited to, one or any combination of peak heating temperature, heating rate, and total heating time, so as to achieve a target degree of heating of the nebulized charged particle droplets. Such one or more charged particle droplet heating parameters will generally be dependent upon a number of different selectable parameters of the system 10 including, for example, but not limited to, one or more electrical parameters of the time-varying electric field 26 such as the frequency, shape, duty cycle, and peak amplitude of the time-varying electric field 26, one or more physical parameters of the system 10, such as the length of the electric field region 25 in which the charged particle droplets are exposed to the time-varying electric field 26, e.g., the distance D1, and one or more temporal parameters of the system 10, such as the time spent by the charged particle droplets moving through the electric field region 25, i.e., the amount of time that the charged particle droplets are exposed to the time-varying electric field 26, which will generally be dependent upon the flow rate of, and distance traveled by, the nebulized charged particles through the electric field region 25. In this regard, nebulized charged particles, i.e., charged particle droplets, are controllably heated with the time-varying electric field 26 by selecting one or more of the electrical parameters of the time-varying electric field 26, one or more of the physical parameters of the system 10, and/or one or more of the temporal parameters of the system 10 so as to achieve a target degree of heating of the nebulized charged particles.

**[0060]** In some example applications, the target degree of heating of the nebulized charged particles may be or include the condition that exposure of the charged particle droplets to the time-varying electric field 26 will result in complete evaporation of the charged particle droplets. In such applications, one or more of the electrical, physical, and/or temporal parameters will be selected so as to cause nebulized charged particles passing through the resulting electric field region 25 with the resulting time-varying electric field 26 established therein to be completely evaporated. In other example applications, the target degree of heating of the nebulized charged particles may be or include the condition that exposure of the charged particle droplets to the time-varying electric field 26 will result in some amount of heating of the charged particle droplets but without complete evaporation of the charged particle droplets. In such applications, one or more of the electrical, physical, and/or temporal parameters will be selected so as to cause nebulized charged particles passing through the resulting electric field region 25 with the resulting time-varying electric field 26 established therein to be heated but not completely evaporated.

**[0061]** In still other example applications in which the sample solution is or contains at least one protein, the target degree of heating of the nebulized charged particles may be or include the condition that exposure of the charged particle droplets to the time-varying electric field 26 will result in partial or complete unfolding of one or more proteins. In such applications, one or more of the electrical, physical, and/or temporal parameters will be selected so as to cause nebulized charged particles passing through the resulting electric field region 25 with the resulting time-varying electric field 26 established therein to be heated sufficiently to cause one or more proteins in the charged particle droplets to partially or fully unfold. Other example applications will occur to those skilled in the art, and it will be understood that such other applications are intended to fall within the scope of this disclosure.

**[0062]** Referring now to Fig. 2, an embodiment is shown of the system 10 of Fig. 1 provided in the form of a field-enabled capillary vibrating sharp-edge spray ionization (FE-CVSSI) system 10'. In the illustrated embodiment, the charged particle source 12 includes a container 40 containing the sample solution 42 therein. The container 40 may be any type of container configured to carry the sample solution 42. In some embodiments, a pump 45 is included to pump the sample solution 42 out of the container 40. In the example embodiment illustrated in Fig. 2, the container 40 is depicted in the form of a syringe. In some such embodiments, the syringe 40 may include a pump, and in other embodiments the system 10' includes the pump 45 separate from the syringe 40 to pump the sample solution 42 from the syringe 40. In any case, the pump 45 (or syringe pump) may illustratively be programmable or controllable by the processor 30 to pump out the sample solution 42 at a selected flow rate. One example flow rate, which should not be considered limiting in any way, is 1-2 micro-liters per minute (μL/min).

**[0063]** The container 40 illustratively includes an outlet fluidly coupled to one end 46A of a capillary 44, as shown in Fig. 2. A portion of the capillary 44 adjacent to an opposite end 46B of the capillary 44 than the end 46A defines an emitter 46 having an emitter tip with an orifice at the end 46B that defines a charged particle outlet orifice 14 of the charged particle source 12. In one example embodiment, which should not be considered limiting in any way, the capillary 44 has an outer diameter of 360 micro-meters (μm), and an inner diameter of 100 μm, and the emitter 46 is illustratively pulled using a conventional P20000 micropipette puller to obtain an emitter tip 46B with a charged particle outlet orifice 14 of approximately 10-20 μm.

**[0064]** A substantially rigid glass slide or glass side coverslip 48 is provided, and the emitter 46 of the capillary 44 is affixed to a top surface 48A of the glass slide 48 adjacent to one edge 48B of the glass slide 48, although in alternate embodiments the emitter 46 may be affixed to the bottom surface of the glass slide 48 opposite the top surface 48A. Illustratively, the emitter 46 is affixed to the glass slide 48 using a conventional adhesive 50 and/or other conventional bonding medium or material.

**[0065]** A conventional piezoelectric transducer 52 is also affixed via an adhesive or other bonding medium or material 54 to the top surface 48A of the glass slide 48 adjacent to an opposite edge 48C of the glass slide 48, although in alternate embodiments the transducer 52 may be affixed to the glass slide 48 anywhere along the top surface 48A or the bottom surface of the glass slide 48. In the illustrated embodiment, a single piezoelectric transducer 52 is affixed to the glass slide 48, although in alternate embodiments two or more piezoelectric transducers may be affixed to the glass slide 48. As described more fully below, the piezoelectric transducer 52 is controllably operated to induce vibrations in the glass slide 48, which vibrations are then transferred to the emitter 46 also affixed to the glass slide 48. In this regard, the piezoelectric transducer 52 represents only one example of a device or devices which may be used to induce vibrations in the rigid, glass slide 48, and it will be understood that one or more other conventional resonant transducers or other transducers configured to convert electrical energy to mechanical energy in the form of vibration(s) may be alternatively or additionally used to induce such vibrations in the glass slide 48.

**[0066]** In the embodiment illustrated in Fig. 2, the voltage source 16 is illustratively provided in the form of a conventional amplifier 56, a conventional waveform generator 60, and a conventional DC power supply 64. In the illustrated embodiment, a signal output of the amplifier 56 is electrically connected, via wires 58, to a signal input of the transducer 52, and a signal input of the amplifier 56 is electrically connected to a signal output of the waveform generator 60 via wires 62. The waveform generator 60 is illustratively operable to produce waveforms in the radio frequency (RF) range, although the waveform generator 60 may in alternate embodiments be configured to produce waveforms outside of the RF range. In the embodiment illustrated in Fig. 2, the amplifier 56 and the waveform generator 60 are separate components, although in alternate embodiments the amplifier 56 and the waveform generator 60 may be combined into a single component. In any case, the waveform signal produced by the waveform generator 60 is applied to the piezoelectric transducer 52 through the amplifier 56. With the transducer 52 and the emitter 46 of the capillary 44 both affixed to the glass slide 48, mechanical vibrations of the transducer 52 produced by the transducer 52 in response to the waveform signal produced by the waveform generator 60 are transmitted via the glass slide 48 to the emitter 46 of the capillary 44. The mechanical vibrations of the emitter 46, in turn, nebulize the sample solution 42 near the emitter tip 46B (e.g., provided thereto via operation of the pump 45 or other pump carried by the container 40) to produce a spray or plume 70 of analyte ions via the charged particle outlet orifice 14 of the emitter 46.

**[0067]** In one example embodiment, which should not be considered limiting in any way, the waveform generator 60 is a Tektronix® AFG-1062 waveform generator, and the amplifier 56 is a Krohn-Hite 7500, and in one example of this embodiment the waveform generator 60 and the amplifier 56 are illustratively configured such that the emitter 46 of the capillary 44 produces plumes 70 of charged particle droplets from the sample solution 42 in a frequency range of 90-100 kHz with RF waveform amplitudes of 5-12 $V_{P-P}$. It will be understood, however, that other conventional waveform generators and/or amplifiers may be used, and that in any embodiment the waveform generator 60 and amplifier 56 may be configured such that the emitter 46 of the capillary 44 produces plumes 70 of charged particle droplets from the sample solution 42 in a frequency range outside of 90-100 kHz and/or with waveform amplitudes outside of the 5-12 $V_{P-P}$ range.

**[0068]** The FE-CVSSI system 10' further includes the DC power supply 64 configured to produce a DC output voltage $V_{DC}$. The DC voltage output of the power supply 64 is electrically connected to a wire 66, e.g. a platinum wire, which is affixed to the outer surface of the capillary 44 or to the outer surface of the emitter 46, e.g., via a conventional adhesive or other bonding medium or material. For CVSSI operation of the system 10', the DC power supply 64 is turned off so that no DC electric field is applied to the sample solution 42 moving through the capillary 44 and the emitter 46. For FE-CVSSI operation of the system 10', the DC power supply 64 is set or controlled to produce a DC voltage, $V_{DC}$, to induce a corresponding electric field in the capillary 44 and/or the emitter 46. In embodiments in which the ions are to be positively charged, the magnitude of $V_{DC}$ will be a positive value so as to orient the electric field to direct positively charged ions out of the charged particle outlet orifice 14 of the emitter 46, and in embodiments in which the ions are to be negatively charged, the magnitude of $V_{DC}$ will be negative so as to orient the electric field to direct negatively charged ions out of the charged particle outlet orifice 14 of the emitter 46.

**[0069]** Referring now to Fig. 3, example oscillating movement of the emitter tip 46B, induced by the mechanical vibrations transmitted from the transducer 52 through the glass slide 48 to the emitter 46 in response to the waveform signal produced by the waveform generator 60, is shown. The position "A" of the emitter tip 46B represents the position of the emitter tip 46B at which the emitter tip 46B, and thus the charged particle outlet orifice 14 of the emitter 46, are aligned, i.e., collinear, with a central axis 18A which bisects the charged particle inlet 18 of the charged particle analysis instrument or system 20. The position "A" illustratively represents the position of the emitter tip 46B, and thus the charged particle outlet orifice 14 of the emitter 46, in the absence of vibrations induced in the glass slide 48 by the AC (e.g., RF) waveform generator 60, or at the zero crossing of the AC (e.g., RF) waveform generated by the waveform generator 60. The position "B" of the emitter tip 46B represents the position of the emitter tip 46B to which the emitter tip 46B, and thus the charged particle outlet orifice 14 of the emitter 46, are moved by the vibrations induced in the glass slide 48 at the positive peak of the AC (e.g., RF) waveform generated by the waveform generator 60. The position "C" of the emitter tip 46B similarly represents the position of the emitter tip 46B to which the emitter tip 46B, and thus the charged particle outlet orifice 14 of the emitter 46, are moved by the vibrations induced in the glass slide 48 at the negative peak of the AC (e.g., RF) waveform generated by the waveform generator 60. In response to the vibrations induced in the glass slide 48 by the AC (e.g., RF) waveform generated by the waveform generator 60, the emitter tip 46B, and thus the charged particle outlet orifice 14 of the emitter 46, oscillate between the two positions “B” and “C.”

**[0070]** Referring now to Fig. 4, an example AC (e.g., RF) electric field 26’ induced in the electric field region 25 of Fig. 3 between the charged particle outlet orifice 14 of the emitter 46 and the charged particle inlet 18 of the charged particle analysis instrument or system 20 is shown. The time-varying electric field 26’ illustratively results from the combination of the oscillating movement of the emitter tip 46B, and thus of the charged particle outlet 14 of the emitter 46, illustrated in Fig. 3 and just described, and the DC voltage, $V_{DC}$, produced by the DC power supply 64 of Fig. 2. As illustrated by example in Fig. 4, a peak positive electric field of approximately 700 V/cm occurs at the position “B” of the emitter tip 46B depicted in Fig. 3, and a peak negative electric field of approximately -1300 V/cm occurs at the position “C” of the emitter tip 46B depicted in Fig. 3.

**[0071]** In some embodiments of the system 10’, the processor 30 may control operation of one or more of the amplifier 56, the waveform generator 60, the pump 45 (or pump carried by the container 40), and the DC power supply 64. In some embodiments, control outputs of the processor(s) 30 is/are electrically connected to one or more control inputs of the amplifier 56 via any number, M, of signal paths, where M may be any positive integer. In some embodiments, control outputs of the processor(s) 30 is/are electrically connected to one or more control inputs of the waveform generator 60 via any number, N, of signal paths, where N may be any positive integer. In some embodiments, control outputs of the processor(s) 30 is/are electrically connected to one or more control inputs of the DC power supply 64 via any number, P, of signal paths, where P may be any positive integer. In embodiments which include the processor(s) 30, the one or more memory devices 32 illustratively has/have stored therein instructions which are executable by the processor(s) 30 to cause the processor(s) 30 to control the amplifier 56, the waveform generator 60, the pump 45, and the DC power supply 64, as described herein. In other embodiments, one or more of the amplifier 56, the waveform generator 60, the pump 45, and the DC power supply 64 may be manually controllable or programmable, and in such embodiments, the role of the processor(s) 30 and memory device(s) 32 may be correspondingly reduced or omitted altogether.

**[0072]** As briefly described above, the time-varying electric field 26' established in the electric field region 25 between the charged particle outlet 14 of the emitter tip 46B and the charged particle inlet 18 of the charged particle analysis instrument or system 20 is illustratively established so as to controllably heat the charged particle droplets in the spray or plume 70 of charged particle droplets. As also described above, the amount or degree of such controlled heating of the charged particle droplets in the plume 70 of charged particle droplets will generally be a function of one or more electrical parameters of the time-varying electric field 26', one or more physical parameters of the system 10', and/or one or more temporal parameters of the system 10'. In the examples depicted in Figs. 5-9 which follow, the frequency, shape, duty cycle, and peak-to-peak voltage applied to the transducer 52 to induce vibrations in the glass slide 48 are illustratively held constant, e.g., by controlling the waveform generator 60 to produce sinusoidal, 50% duty cycle, approximately 10 volts peak-to-peak RF waveforms of approximately 100 kHz, and the length D1 of the electric field region 25 is approximately 3 millimeters. The flow rate of the charged particle droplets in the plume 70 of charged particle droplets passing through the time-varying electric field 26' will generally be a function of the flow rate of the plume 70 exiting the charged particle outlet 14 of the emitter tip 46B and the pressure differential between the ambient pressure about the emitter tip 46B, e.g., atmospheric pressure, and the vacuum conditions in the region 22 of the charged particle analysis instrument or system 20 established by the pump 24. The magnitude of a positive DC voltage, $V_{DC}$, produced by the DC power supply 64 is illustratively the sole variable of the selectable parameters, and is varied to correspondingly vary the peak-to-peak magnitude of the RF electric field 26' induced in the electric field region 25 to demonstrate the effects of varying amounts or degrees of heating of the charged particle droplets in the plume 70 of charged particle droplets emitted from the charged particle outlet 14 of the emitter tip 46B. It will be understood that similar results may be obtained by maintaining $V_{DC}$ at a constant magnitude, and varying one or more others of the electrical parameters of the time-varying electric field 26', one or more physical parameters of the system 10' such as the length or distance D1, and/or one or more temporal parameters of the system 10', such as the time spent by the charged particle droplets in the time-varying electric field 26', any one or more of which may affect the amount or degree of heating of the charged particle droplets in the plume 70 of charged particle droplets emitted from the charged particle outlet 14 of the emitter tip 46B.

**[0073]** Referring now to Fig. 5, a plot 200 is shown of FE-CVSSI operation of the system 10' illustrated in Fig. 2, and depicts charge state fraction vs. $V_{DC}$ for a sample solution of Ubiquitin. In Figs. 6-9, plots are shown of relative abundance vs. mass-to-charge ratio, as produced by an ion separation instrument in the form of a conventional mass spectrometer. The four different $V_{DC}$ conditions highlighted in Fig. 5 are used in Figs. 6-9. In the example illustrated in Fig. 5, $V_{DC}$ is ramped between 0 volts and 1 kV. As depicted in Fig. 5 and also depicted in Fig. 6 which shows relative abundance vs. mass-to-charge ratio under $V_{DC1}$ conditions ($V_{DC}$ = 0), the charge states +2 through +6 are present under CVSSI conditions, with the charge states +3, +4 and +5 dominating in respective order of relative abundance, as shown in Fig. 6. As further depicted in Fig. 5 and in Fig. 9 which shows relative abundance vs. mass-to-charge ratio under $V_{DC4}$ conditions ($V_{DC}$ = 1 kV), the charge states +3 through +9 are present, with the charge states +6, +5 and +4 dominating in respective order of relative abundance.

**[0074]** As depicted in Figs. 5, 7, and 8, in contrast, higher charge states emerge between the $V_{DC1}$ and $V_{DC4}$ conditions due to unfolding of the respective protein ions. In Fig. 7, for example, which shows relative abundance vs. mass-to-charge ratio under $V_{DC2}$ conditions ($V_{DC}$ = 350 volts), charge states +3 through +10 are present, wherein the charge states +7 through +10 are present in substantially greater abundances than in either of the $V_{DC1}$ and $V_{DC4}$ conditions. Similarly in Fig. 8, which shows relative abundance vs. mass-to-charge ratio under $V_{DC3}$ conditions ($V_{DC}$ = 600 volts), charge states +3 through +10 are again present, and the charge states +7 through +10 are likewise present but in substantially greater abundances than in either of the $V_{DC1}$ and $V_{DC4}$ conditions. Thus, under $V_{DC}$ conditions between $V_{DC}$ = 0 volts and $V_{DC}$ = 1 kV, high charge states of Ubiquitin are present which are either undetectable or present only in very low abundances under the $V_{DC}$ = 0 volts and $V_{DC}$ = 1 kV, conditions, i.e., which are either undetectable or present only in very low abundances outside of the electric field range induced in the $V_{DC}$ range $0 < V_{DC} < 1$ kV. In this range, the Ubiquitin proteins undergo various states of unfolding due to heating of the RF-generated charged particle droplets, while unfolding has not been observed outside of this range.

**[0075]** Referring now to Fig. 10, another embodiment is shown of the system 10 of Fig. 1 provided in the form of a system 10'' including another embodiment of an electric field generation device 28 for controllably heating the nebulized charged particles prior to charged particle analysis. In the illustrated embodiment, the system 10'' is identical in many respects to the system 10 illustrated in Fig. 1 and described above, and like numbers are therefore used to identify like components. The charged particle source 12, for example, may be or include any of the charged particle sources described above with respect to Figs. 1-9, configured and operable to produce and expel a plume 80 of charged particle droplets from the charged particle outlet 14 as depicted by example in Fig. 10. The charged particle analysis instrument or system 20, as another example, may be or include any one or combination of instruments and/or devices as described above.

**[0076]** In the embodiment illustrated in Fig. 10, the time-varying electric field generation device 28 is provided in the form of opposed, electrically conductive sheets or plates 82 and 84 spaced apart from one another to form the electric field region 25 therebetween. The voltage source 16' is illustratively configured to produce an AC voltage, AC, as described above with respect to Figs. 1-9, which is applied to and between the plates 82, 84 to establish the time-varying electric field 26 within the electric field region 25 as also described above.

**[0077]** In the illustrated embodiment, the plates 82, 84 are positioned symmetrically about the central axis 18A which bisects the charged particle inlet 18 of the charged particle analysis instrument or system 20, although in alternate embodiments the plates 82, 84 may not be so symmetrically positioned. Also in the illustrated embodiment, the distance D1 is depicted consistently with Fig. 1 as the distance between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet 18 of the charged particle analysis instrument or system 20. The length of the electric field region 25 defined by the plates 82, 84 is defined by the respective lengths D2 of the plates 82, 84. In the illustrated embodiment, D2 is less than D1 (D2 < D1), and in such embodiments the difference between D1 and D2 (D1 – D2) or the ratio of D1 to D2 (D1/D2) may be another physical parameter of the system 10'' having a bearing on the amount or degree of heating of the charged particle droplets traveling between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet 18 of the charged particle analysis instrument or system 20. In alternate embodiments, D2 may be equal to D1 as illustrated by example in Fig. 1.

**[0078]** Further still in the illustrated embodiment, the plates 82, 84 are shown disposed above and below the central axis 18A so as to define a vertical distance D3 between the plates 82, 84. In alternate embodiments, the plates 82, 84 may be disposed on either side of the central axis 18A so as to define a horizontal distance D3 between the plates 82, 84. In still other embodiments, the plates 82, 84 may be disposed at an angle relative to the vertical or horizontal axis. In any case, the distance D3 between the plates 82, 84 may be yet another physical parameter of the system 10" which may have a bearing on the amount or degree of heating of the charged particle droplets traveling between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet 18 of the charged particle analysis instrument or system 20. In some embodiments, the plates 82, 84 may be planar, e.g., flat, such that the inwardly-facing surfaces of the plates 82, 84 are parallel with one another. In some alternate embodiments, either or both of the plates 82, 84 may be non-planar.

**[0079]** In some embodiments of the system 10" illustrated in Fig. 10, the end of the electric field region 25 adjacent to the charged particle outlet 14 of the charged particle source 12 may be open; that is, the ends of the plates 82, 84 adjacent to the charged particle source 12 may be open to the charged particle source 12. The opposite end of the electric field region 25 adjacent to the charged particle inlet 18 of the charged particle analysis instrument or system 20 may likewise be open. In some alternate embodiments, an electrically conductive gate or grid 86 may be positioned between the electric field region 25 and the charged particle outlet 14 of the charged particle source 12, e.g., adjacent to and vertically between the respective ends of the plates 82, 84, and a DC voltage outlet DC1 of the voltage source 16' may be electrically connected to the gate or grid 86 as depicted in dashed-line representation in Fig. 10. In such embodiments, the DC voltage DC1 may be controlled to selectively allow charged particle droplets in the plume 80 of charged particle droplets to enter the electric field region 25, and to selectively reject or prevent charged particle droplets in the plume 80 of charged particle droplets from entering the electric field region 25.

**[0080]** Alternatively or additionally, an electrically conductive gate or grid 88 may be positioned between the electric field region 25 and the charged particle inlet 18 of the charged particle analysis instrument or system 20, e.g., adjacent to and vertically between the respective ends of the plates 82, 84, and a DC voltage outlet DC2 of the voltage source 16' may be electrically connected to the gate or grid 88 as also depicted in dashed-line representation in Fig. 10. In such embodiments, the DC voltage DC2 may be controlled to selectively allow charged particle droplets moving through the electric field region 25 to exit the electric field region 25 and enter the charged particle inlet 18 of the charged particle analysis instrument or system 20, and to selectively reject or prevent charged particle droplets moving through the electric field region 25 from exiting the electric field region 25 so that such charged particle droplets do not enter the charged particle inlet 18 of the charged particle analysis instrument or system 20.

**[0081]** In some embodiments which include both of the gates or grids 86, 88, the DC voltages DC1 and DC2 may be controlled to selectively establish an electric field within the electric field region 25, e.g., in the direction 27 for positively charged particle droplets as illustrated by dashed-line representation in Fig. 10, or in the opposite direction for negatively charged particle droplets. In embodiments in which the charged particle source 12 and the time-varying electric field generation device 28 are both subject to the same pressure conditions, e.g., atmospheric pressure in the embodiment illustrated in Fig. 10, such a DC electric field 27 may be used, for example, as a motive force to move the charged particle droplets in the plume 80 of charged particle droplets generated by the charged particle source 12 through the electric field region 25 of the time-varying electric field generation device 28 and into the charged particle analysis instrument or system 20 via the charged particle inlet 18. In such embodiments, the flow rate of charged particle droplets through the electric field region 25 of the time-varying electric field generation device 28 will be dependent upon the magnitude of the DC electric field 27.

**[0082]** In some embodiments, a DC output, DC3, of the voltage source 16' may be electrically connected to and across the plates 82, 84 as illustrated by dashed-line representation in Fig. 10. In such embodiments, the magnitude and polarity of the DC voltage DC3 may be controlled to selectively create a DC electric field within the electric field region 25 for offsetting, if and as desired, the AC electric field established in the electric field region 25 by the AC voltage produced at the AC output of the voltage source 16'.

**[0083]** In the embodiment illustrated in Fig. 10, one or more of the electrical parameters of the time-varying electric field 26, one or more of the physical parameters of the system 10'', and/or one or more of the temporal parameters of the system 10'' may be selected and/or controlled as described above so as to controllably heat the charged particle droplets in the spray or plume 80 of charged particle droplets exiting the charged particle outlet 14 of the charged particle source 12 to a target degree of heating of the nebulized charged particles, as described above. The amount or degree of such controlled heating of the charged particle droplets in the plume 80 of charged particle droplets exiting the charged particle outlet 14 of the charged particle source 12 and entering the charged particle inlet 18 of the charged particle analysis instrument or system 20 will generally be a function of such physical, electrical, and temporal parameters, as also described above. In the system 10'' illustrated by example in Fig. 10, the one or more electrical parameters of the time-varying electric field 26 may be any one of more such parameters described above. The one or more physical parameters of the system 10'' may be or include, but are not limited to, any one or more of the distances or lengths D1, D2, D3, one or more ratios of any combination of D1, D2, D3, and the positioning of the time-varying electric field generation device 28 within the space D1 (e.g., the length of the gap between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet of the electric field generation device 28, and the length of the gap between the charged particle outlet of the electric field generation device 28 and the charged particle inlet 18 of the charged particle analysis instrument or system 20). The one or more temporal parameters of the system 10'' may illustratively be or include, but are not limited to, the time spent by the charged particle droplets moving through the time-varying electric field 26, the time spent by the charged particle droplets moving through one or more portions of the region of D1 not occupied by the time-varying electric field generation device 28, and/or the time spent by the charged particle droplets moving through the entire region D1 between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet of the charged particle analysis instrument or system 20.

**[0084]** With respect to the one or more temporal parameters, the flow rate of charged particle droplets between the charged particle outlet 14 of the charged particle source 12 and the charged particle inlet 18 of the charged particle analysis instrument or system 20 may depend on a number of different factors relating to the particular configuration of the system 10”. For example, in embodiments in which D1 = D2, the flow rate of charged particle droplets between the charged particle source 12 and the charged particle analysis instrument or system 20 will be defined by the flow rate of charged particle droplets through the electric field region 25 defined by the time-varying electric field generation device 28. In embodiments which do not include the gates or grids 86, 88, the flow rate of charged particle droplets through the electric field region 25, with D1 = D2, will depend primarily on the flow rate of charged particles exiting the charged particle outlet 14 of the charged particle source 12, the pressure differential between the electric field region 25 and the region 22 of the charged particle analysis instrument or system 20 and the length D2 of the electric field region 25. In embodiments which do include the gates or grids 86, 88, the flow rate of charged particle droplets through the electric field region 25 will further depend on the magnitude and direction of the DC electric field 27 established between the gates or grids 86, 88. In some such embodiments, the DC electric field 27 may dominate the flow rate of charged particle droplets through the electric field region 25 such that the flow rate of charged particle droplets exiting the charged particle outlet 14 of the charged particle source 12 and the pressure differential between the electric field region 25 and the region 22 of the charged particle analysis instrument or system 20 may be negligible.

**[0085]** In embodiments in which D1 is greater than D2, the flow rate of charged particle droplets between the charged particle source 12 and the charged particle analysis instrument or system 20 will generally be defined by a combination of (i) the flow rate of charged particle droplets through the region of D1 between the charged particle outlet 14 of the charged particle source and the inlet or entrance to the electric field region 25 of the time-varying electric field generation device 28 (FR1), (ii) the flow rate of charged particle droplets through the electric field region 25 (FR2), and (iii) the flow rate of charged particle droplets through the region of D1 between the outlet of the electric field region 25 of the time-varying electric field generation device 28 and the charged particle inlet 18 of the charged particle analysis instrument or system 20 (FR3). In some such embodiments, the target degree of heating of the charged particle droplets may further take into account potential cooling of the charged particle droplets over the portion of D1 between the outlet of the time-varying electric field generation device 28 and the charged particle inlet 18 of the charged particle analysis instrument or system 20.

**[0086]** In any case, FR1 will typically depend at least on the flow rate of charged particles exiting the charged particle outlet 14 of the charged particle source 12 and the distance between the charged particle outlet 14 of the charged particle source 12 and the inlet or entrance to the electric field region 25 of the time-varying electric field generation device 28. FR3 will typically depend at least on the flow rate of charged particles exiting the electric field region 25 of the time-varying electric field generation device 28, the distance between the outlet of the time-varying electric field generation device 28 and the charged particle inlet 18 of the charged particle analysis instrument or system 20, and the pressure differential between the pressure of the portion of D1 between the outlet of the time-varying electric field generation device 28 and the charged particle inlet 18 of the charged particle analysis instrument or system 20 and the pressure established in the region 22 of the charged particle analysis instrument or system 20 established by the pump 24. In embodiments which do not include the gates or grids 86, 88, FR2 will depend primarily on the flow rate of charged particles exiting the charged particle outlet 14 of the charged particle source 12, the distance between the charged particle outlet 14 and the entrance to the time-varying electric field generation device 28, the length D2 of the electric field region 25 defined by and within the time-varying electric field generation device 28, and the effect, if any, on the pressure differential between the electric field region 25 and the region 22 of the charged particle analysis instrument or system 20 over the distance between the outlet of the time-varying electric field generation device 28 and the charged particle inlet 18 of the charged particle analysis instrument or system 20. In embodiments which do include the gates or grids 86, 88, the flow rate of charged particle droplets through the electric field region 25 will further depend on the magnitude and direction of the DC electric field 27 established between the gates or grids 86, 88.

**[0087]** Referring now to Fig. 11, yet another embodiment is shown of the system 10 of Fig. 1 provided in the form of a system 10''' including the electric field generation device 28 of Fig. 10 positioned within the front end of the charged particle analysis instrument or system 20. In the illustrated embodiment, the system 10''' is identical in structure and operation to the system 10" illustrated in Fig. 10 described above, except that the time-varying electric field generation device 28 is disposed within a stage 90 of the charged particle analysis system 20 which represents the charged particle inlet from outside of the charged particle analysis instrument or system 20 and which is fluidly coupled to the charged particle inlet 18 of the charged particle analysis instrument or system 20. In the embodiment illustrated in Fig. 11, the voltage source 16' of Fig. 10 is depicted as being provided in the form of two voltage sources 16A and 16B. The voltage source 16A is configured to produce one or more voltages for controlling the charged particle source 12, in a conventional manner, to produce charged particle droplets in the form of the plume 80 of charged particle droplets. The voltage source 16B is configured to produce at least the AC voltage for establishing the time-varying electric field within the time-varying electric field generation device 28 as described above with respect to Fig. 10 (and in some embodiments, to also produce the DC voltage(s) DC1, DC2, and/or DC3 as depicted by example in Fig. 10).

**[0088]** The stage 90 illustratively has a charged particle inlet 92 via which charged particle droplets in the plume 80 of charged particle droplets, generated by the charged particle source 12, enter the stage 90. The charged particle droplets in the plume 80 of charged particle droplets entering the stage 90 then enter the time-varying electric field generation device 28 (in which the time-varying electric field 26 is generated as described above), and charged particles exiting the electric field generation device 28 enter the charged particle inlet 18 of the charged particle analysis instrument or system 20.

**[0089]** The stage 90 is fluidly coupled to a conventional pump 94, and the pump 94 is controlled to a pressure that is less than the pressure in the environment of the charged particle source 12, e.g., atmospheric pressure, and that is greater than the pressure established by the pump 24 in the region 22 of the charged particle analysis instrument or system 20. Illustratively, the pressure differential in the differentially pumped stages 90, 22 of the instrument or system 20 serves to move the charged particles through the stage 90 and into the stage or region 22. In such embodiments, the DC voltage output DC3 of the voltage source 16B therefore may not be electrically connected to the electric field generation device 28 as illustrated in Fig. 10 and described above, although some alternate embodiments may include the DC output voltage DC3 electrically connected to the electric field generation device 28 and controlled to produce a DC voltage as described above with respect to Fig. 10. In embodiments in which the electric field generation device 28 includes the gate(s) or grid(s) 86, 88 as described above with respect to Fig. 10, the voltage source 16B may be configured as also described above to apply suitable DC voltage(s) to the gate(s) or grid(s) 86, 88.

**[0090]** Referring now to Fig. 12, still a further embodiment is shown of the system 10 of Fig. 1 provided in the form of a system $10^{IV}$ including the time-varying electric field generation device 28 of Fig. 10 positioned between charged particle analysis and/or processing stages of the charged particle analysis instrument or system 20. The system $10^{IV}$ is illustratively identical in structure and operation to the system 10''' illustrated in Fig. 11, except that a charged particle analysis and/or processing stage 93 of the charged particle analysis instrument or system 20 is positioned upstream of the stage 90 in which the time-varying electric field generation device 28 is disposed, i.e., such that the stage 90 is positioned between the stage 93 and the stage 22 of the charged particle analysis instrument or system 20. The stage 93 illustratively defines a charged particle inlet 91 through which the plume 80 of charged particle droplets, generated by the charged particle source 12, enters the stage 93. Illustratively, the stage 93 may be or include one or any combination of the charged particle analysis instruments and/or charged particle processing instruments or devices described above with respect to Fig. 1, such that the plume 80 of charged particle droplets may be analyzed and/or processed prior to being controllably heated by the time-varying electric field generation device 28 as described above.

**[0091]** The stage 93 is fluidly coupled to a conventional pump 95, and the pump 95 is controlled to a pressure that is less than the pressure in the environment of the charged particle source 12, e.g., atmospheric pressure, and that is greater than the pressure established by the pump 94 in the stage 90 of the charged particle analysis instrument or system 20. Illustratively, the pressure differential in the differentially pumped stages 93, 90, 22 of the charged particle analysis instrument or system 20 serves to move the charged particles sequentially through the stages 93, 90, and 22.

**[0092]** While the disclosure has been illustrated and described in detail in the drawings and foregoing description, such an illustration and description is to be considered as exemplary and not restrictive in character, it being understood that only illustrative embodiments have been shown and described and that all changes and modifications consistent with the disclosure and recited claims are desired to be protected.

**What is claimed is:**

1. A method for controllably heating nebulized charged particles, the method comprising:

configuring a time-varying electric field to heat the nebulized charged particles passing therethrough with a target degree of heating; and

passing the nebulized charged particles through the configured time-varying electric field to heat the nebulized charged particles with the target degree of heating.

2. The method of claim 1, wherein configuring the time-varying electric field comprises selecting at least one electrical parameter of the time-varying electric field which will achieve the target degree of heating of the nebulized charged particles.

3. The method of claim 2, wherein the at least one electrical parameter of the time-varying electric field comprises one or any combination of frequency, shape, duty cycle, and peak amplitude of the time-varying electric field.

4. The method of any of claims 1 through 3, further comprising establishing the configured time-varying electric field within and along an electric field region.

5. The method of claim 4, wherein configuring the time-varying electric field comprises selecting a length of the electric field region which will achieve the target degree of heating of the nebulized charged particles.

6. The method of claim 4, wherein the nebulized charged particles have a flow rate with which the nebulized charged particles pass through the configured time-varying electric field,

and wherein configuring the time-varying electric field comprises selecting either or both of the at least one electrical parameter and the length of the electric field region which will achieve the target degree of heating of the nebulized charged particles taking into account the flow rate of the nebulized charged particles through the configured time-varying electric field.

7. The method of any of claims 1 through 6, wherein configuring the time-varying electric field to heat the nebulized charged particles comprises configuring the time-varying electric field in a manner which controls at least one of peak heating temperature, heating rate, and total heating time of the nebulized charged particles passing through the configured time-varying electric field.

8. The method of any of claims 1 through 7, wherein the target degree of heating comprises complete evaporation of the nebulized charged particles exiting the configured time-varying electric field.

9. The method of any of claims 1 through 7, wherein the target degree of heating comprises incomplete evaporation of the nebulized charged particles exiting the configured time-varying electric field.

10. The method of any of claims 1 through 7, wherein the nebulized charged particles carry at least one protein,

and wherein the target degree of heating comprises at least partial unfolding of the at least one protein.

11. A method of analyzing charged particles, comprising:

generating nebulized charged particles;

passing the generated nebulized charged particles through the configured time-varying electric field of any of claims 1 through 10 to heat the generated nebulized charged particles with the target degree of heating; and

analyzing at least one molecular characteristic of the generated nebulized charged particles exiting the configured time-varying electric field.

12. A method of analyzing charged particles, comprising:

generating nebulized charged particles;

passing the generated nebulized charged particles into a pressure-controlled charged particle analysis instrument;

within the pressure-controlled charged particle analysis instrument, passing the generated nebulized charged particles through the configured time-varying electric field of any of claims 1 through 10 to heat the generated nebulized charged particles with the target degree of heating; and

within the pressure-controlled charged particle analysis instrument, analyzing at least one molecular characteristic of the generated nebulized charged particles exiting the configured time-varying electric field.

13. A method of analyzing charged particles, comprising:

generating nebulized charged particles;

passing the generated nebulized charged particles into a pressure-controlled charged particle analysis instrument;

within the pressure-controlled charged particle analysis instrument, analyzing or processing the generated nebulized charged particles according to a first molecular characteristic;

within the pressure-controlled charged particle analysis instrument, passing the nebulized charged particles, analyzed or processed according to the first molecular characteristic, through the configured time-varying electric field of any of claims 1 through 10 to heat the generated nebulized charged particles with the target degree of heating; and

within the pressure-controlled charged particle analysis instrument, analyzing the generated nebulized charged particles exiting the configured time-varying electric field according to the first or a second molecular characteristic.

14. An apparatus for controllably heating nebulized charged particles, the apparatus comprising:

an electric field region configured to receive the nebulized charged particles therethrough; and

means for establishing a time-varying electric field within the electric field region such that the nebulized charged particles pass through the time-varying electric field, the time-varying electric field configured to heat the nebulized charged particles passing therethrough with a target degree of heating.

15. The apparatus of claim 14, wherein the time-varying electric field is configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring at least one electrical parameter of the time-varying electric field in a manner which will achieve the target degree of heating of the nebulized charged particles,

and wherein the at least one electrical parameter comprises one or any combination of frequency, shape, duty cycle, and peak amplitude of the time-varying electric field.

16. The apparatus of claim 14 or 15, wherein the electric field region has a length in and along which the time-varying electric field is established,

and wherein the time-varying electric field is configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring the length of the electric field region in a manner which will achieve the target degree of heating of the nebulized charged particles.

17. The apparatus of any of claims 14 through 16, wherein the nebulized charged particles have a flow rate with which the nebulized charged particles pass through the electric field region,

and wherein the time-varying electric field is configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring either or both of the at least one electrical parameter and the length of the electric field region which will achieve the target degree of heating of the nebulized charged particles taking into account the flow rate of the nebulized charged particles through the electric field region.

18. The apparatus of any of claims 14 through 17, wherein the time-varying electric field is configured to heat the nebulized charged particles passing therethrough with the target degree of heating by configuring the time-varying electric field in a manner which controls at least one of peak heating temperature,

heating rate, and total heating time of the nebulized charged particles passing through the electric field region.

19. The apparatus of any of claims 14 through 18, wherein the time-varying electric field is configured to heat the nebulized charged particles passing therethrough with the target degree of heating by heating the nebulized charged particles to complete evaporation.

20. The apparatus of any of claims 14 through 18, wherein the time-varying electric field is configured to heat the nebulized charged particles passing therethrough with the target degree of heating by heating the nebulized charged particles in a manner which does not completely evaporate the nebulized charged particles.

21. The apparatus of any of claims 14 through 18, wherein the nebulized charged particles carry at least one protein,

and wherein the time-varying electric field is configured to heat the nebulized charged particles passing therethrough with the target degree of heating by heating the nebulized charged particles in a manner which results in at least partial unfolding of the at least one protein.

22. The apparatus of any of claims 14 through 21, wherein the means for establishing the time-varying electric field within the electric field region further comprises means for generating the nebulized charged particles.

23. The apparatus of claim 22, wherein the means for establishing the time-varying electric field within the electric field region comprises:

a rigid substrate;

a capillary having one end fluidly coupled to a sample solution and an opposite end forming an emitter with an emitter tip defining an orifice therein, a portion of the emitter being affixed to the rigid substrate;

a transducer coupled to the rigid substrate;

a waveform generator electrically coupled to the transducer and configured to produce time-varying waveforms, the transducer responsive to the time-varying waveforms to vibrate the rigid substrate to cause the emitter tip to oscillate;

a pump configured to pump the sample solution through the capillary so as to exit the orifice of the oscillating emitter tip into the electric field region in the form of a plume of the nebulized charged particles; and

a voltage source configured to apply a DC voltage to the emitter tip to induce the time-varying electric field in the plume of the nebulized charged particles exiting the oscillating emitter tip.

24. The apparatus of any of claims 14 through 21, wherein the means for establishing the time-varying electric field within the electric field region comprises:

first and second conductive sheets or plates spaced apart from one another to define the electric field region therebetween; and

at least one voltage source configured to apply a time-varying voltage to and between the first and second sheets or plates to establish the time-varying electric field between the first and second sheets or plates.

25. The apparatus of claim 24, further comprising a charged particle analysis instrument,

and wherein the electric field region is external to the charged particle analysis instrument and positioned adjacent to a charged particle inlet of the charged particle analysis instrument such the nebulized charged particles exiting the electric field region enter the charged particle inlet of the charged particle analysis instrument.

26. The apparatus of claim 24, further comprising a charged particle analysis instrument,

and wherein the electric field region is positioned within the charged particle analysis instrument.

27. The apparatus of claim 25 or claim 26, further comprising a charged particle source configured to generate the nebulized charged particles and supply the nebulized charged particles to the electric field region.

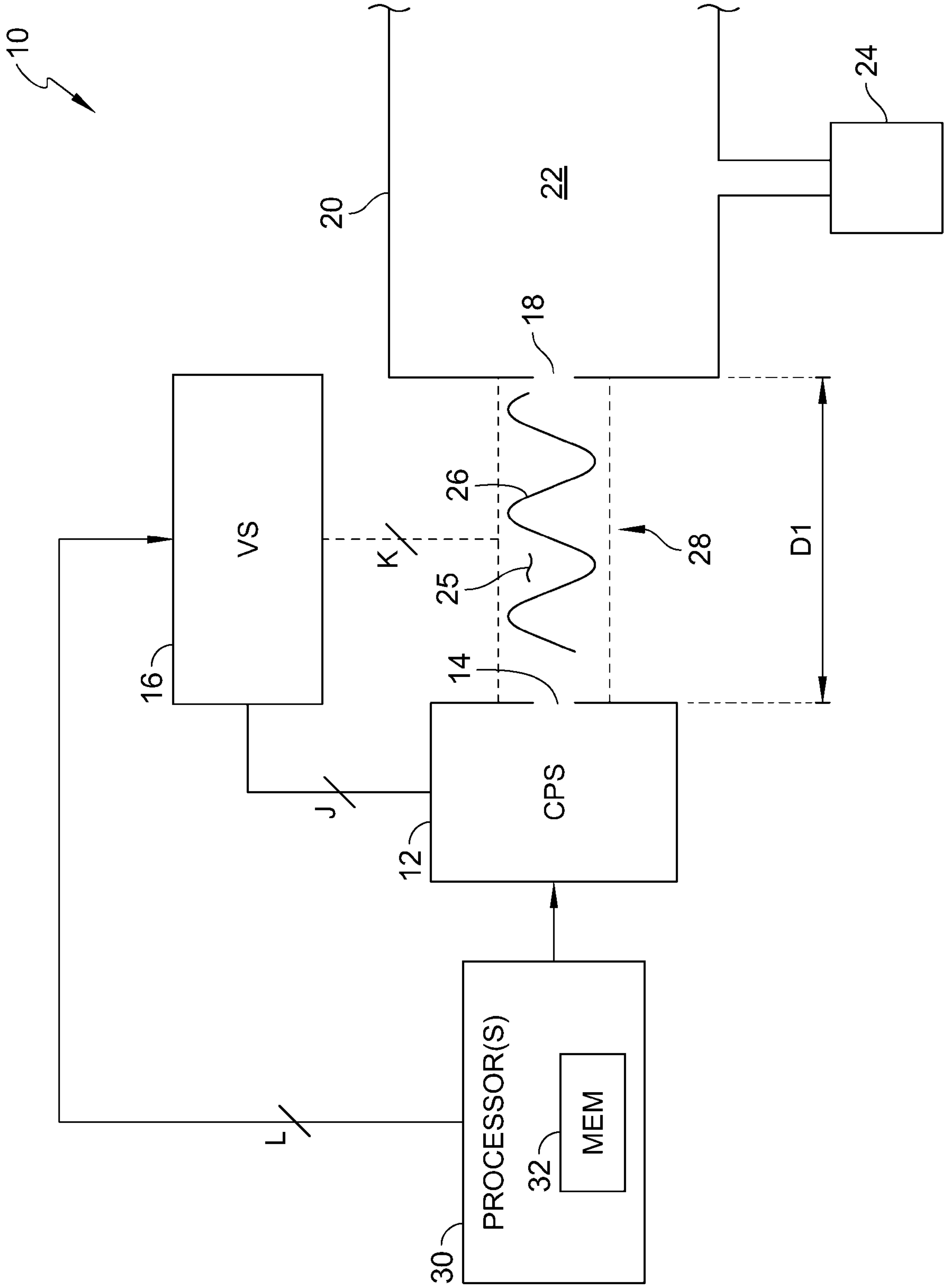



FIG. 1

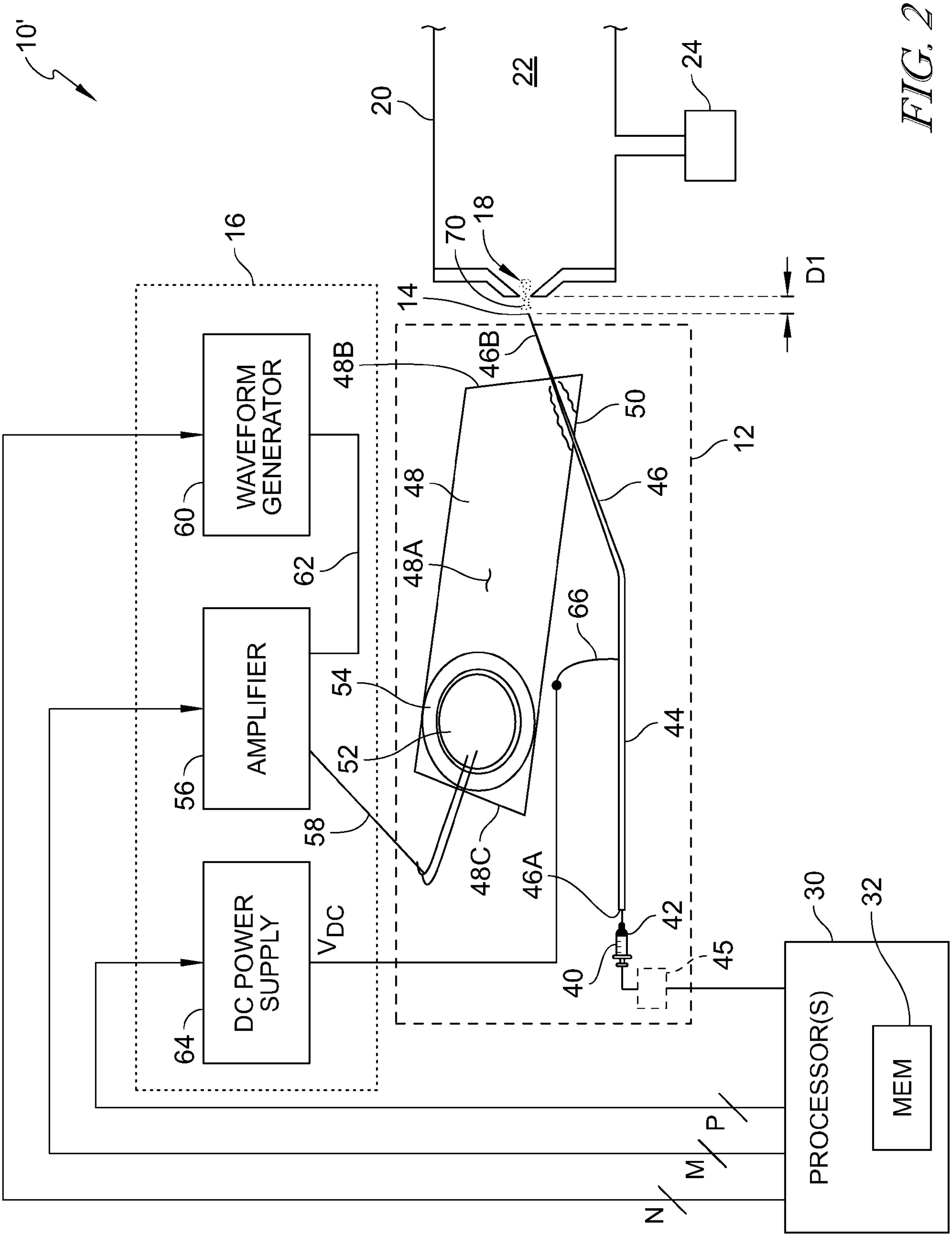

10'
20
22
24
16
70
18
14
D1
48B
46B
50
12
WAVEFORM GENERATOR
60
48
46
48A
62
66
AMPLIFIER
54
44
52
56
58
48C
46A
42
DC POWER SUPPLY
$V_{DC}$
40
45
64
30
32
PROCESSOR(S)
MEM
P
M
N

FIG. 2

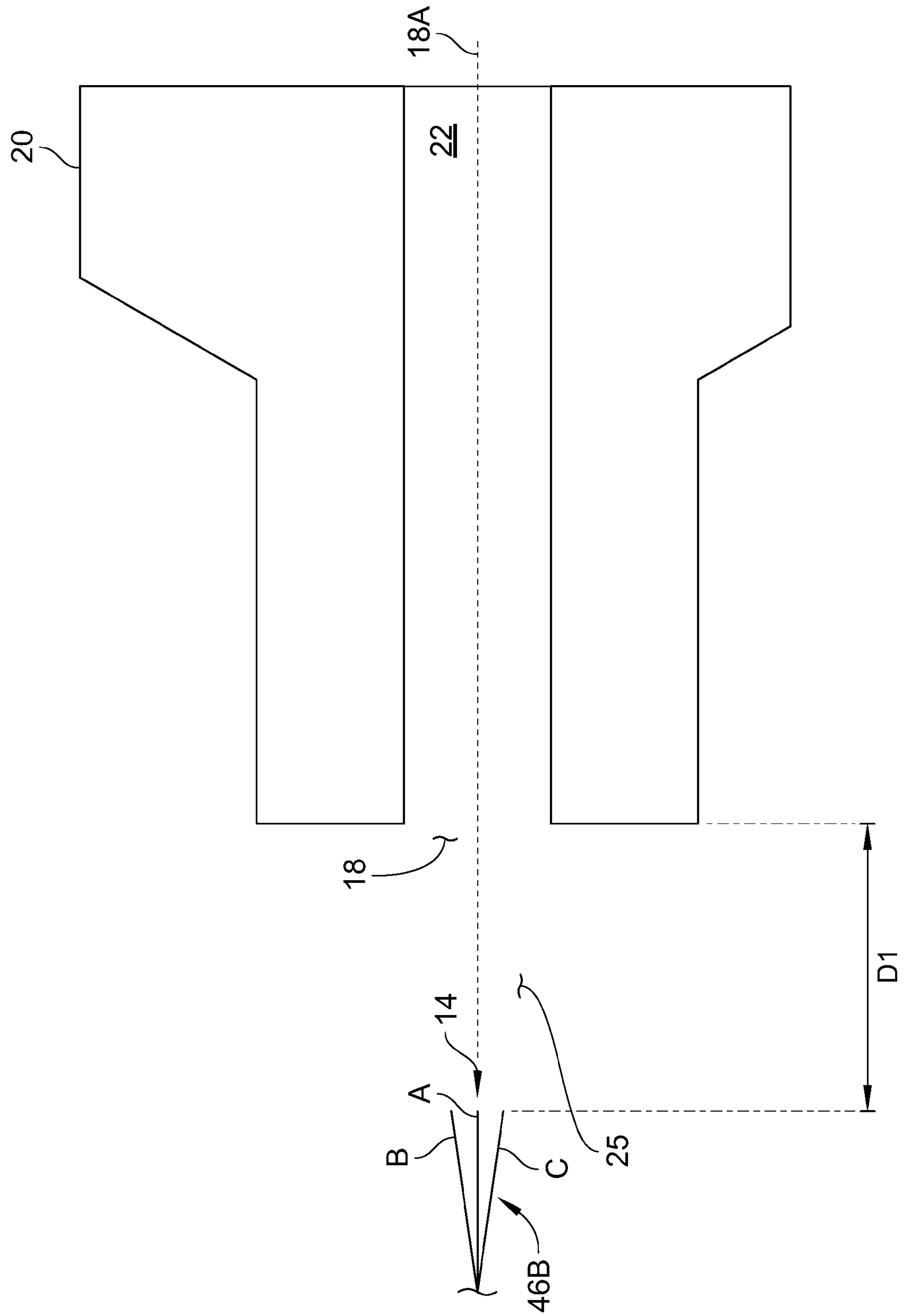



*FIG. 3*

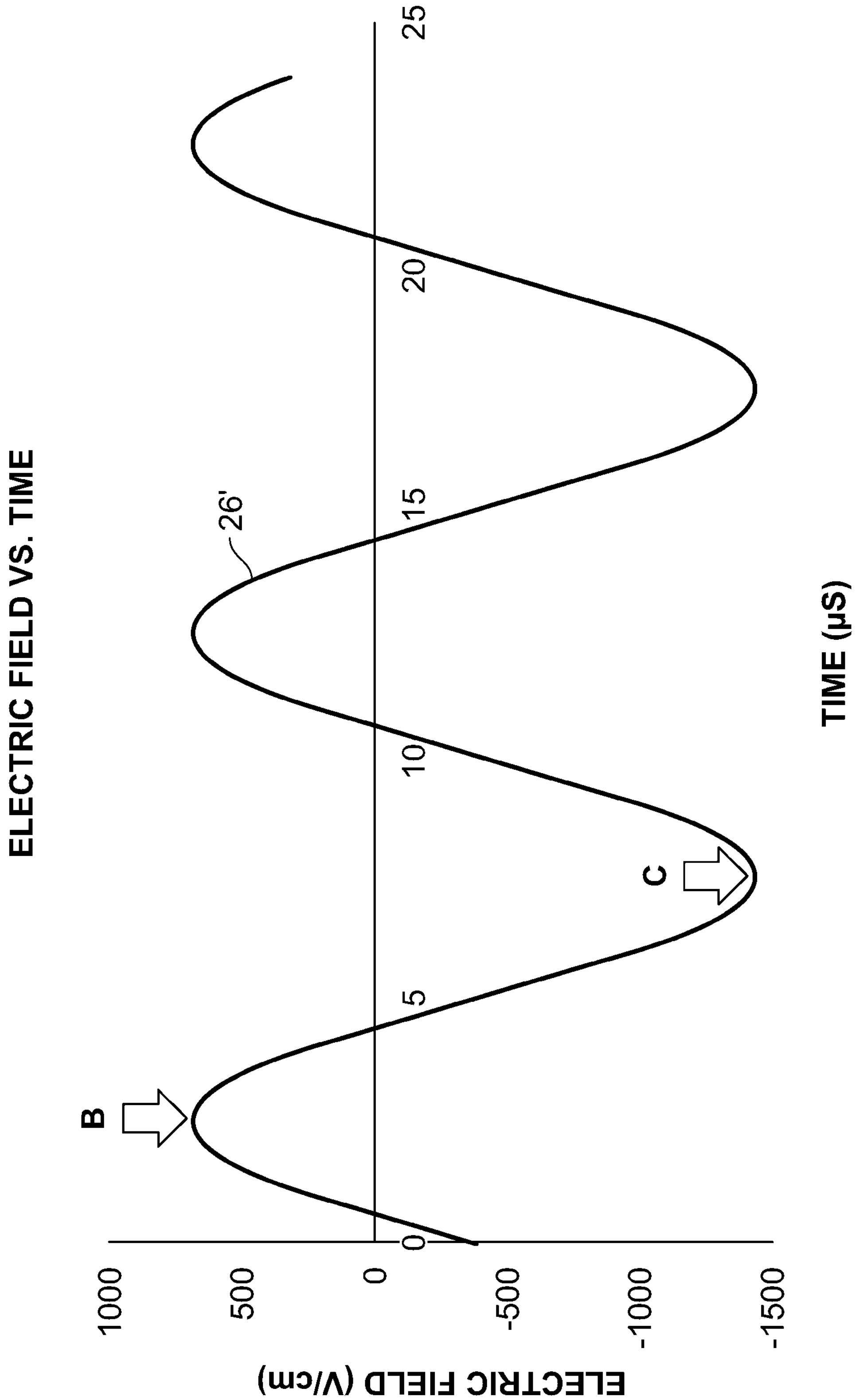

ELECTRIC FIELD VS. TIME
ELECTRIC FIELD (V/cm)
1000
500
0
-500
-1000
-1500
0
5
10
15
20
25
TIME (µS)
B
C
26'

*FIG. 4*

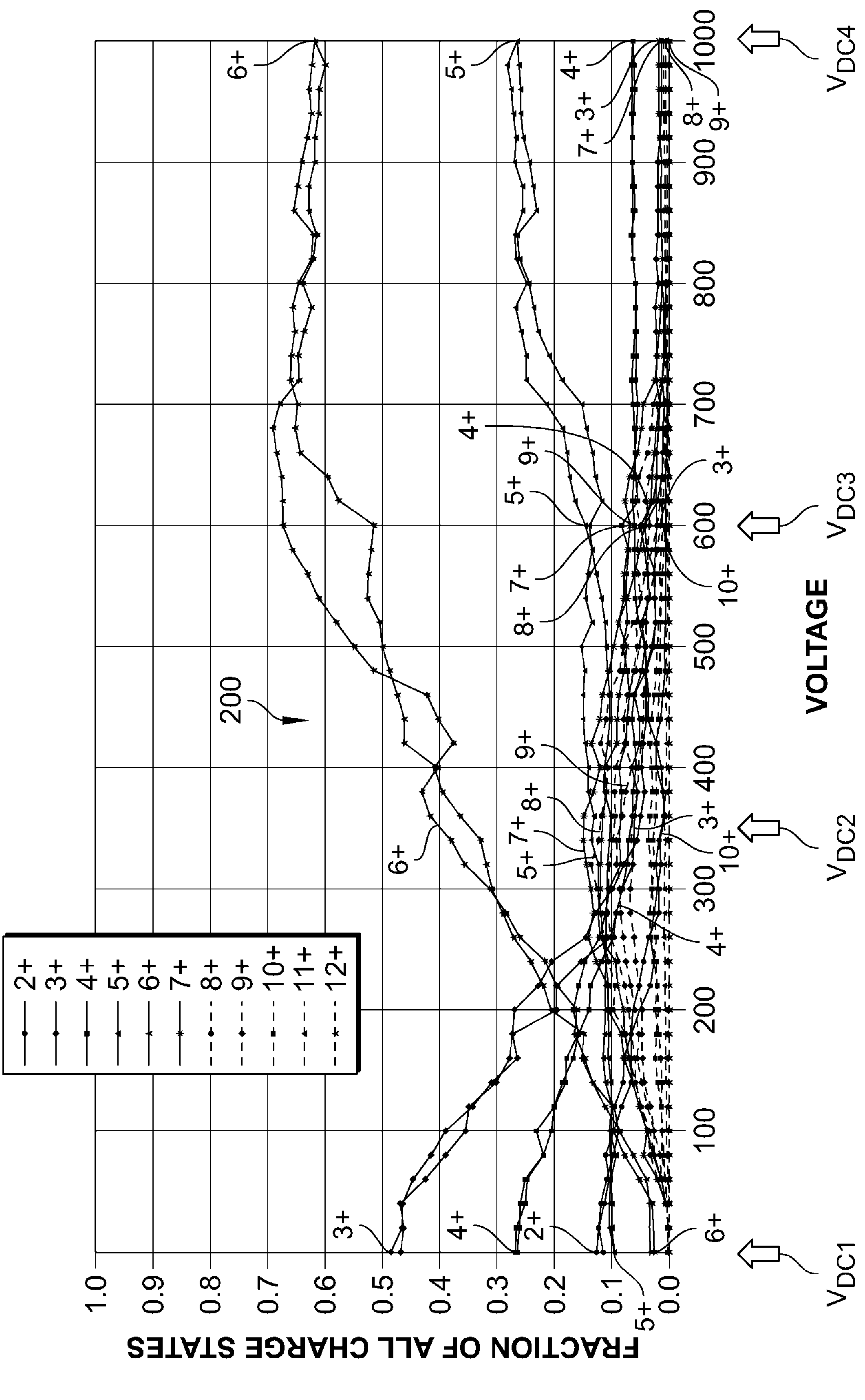
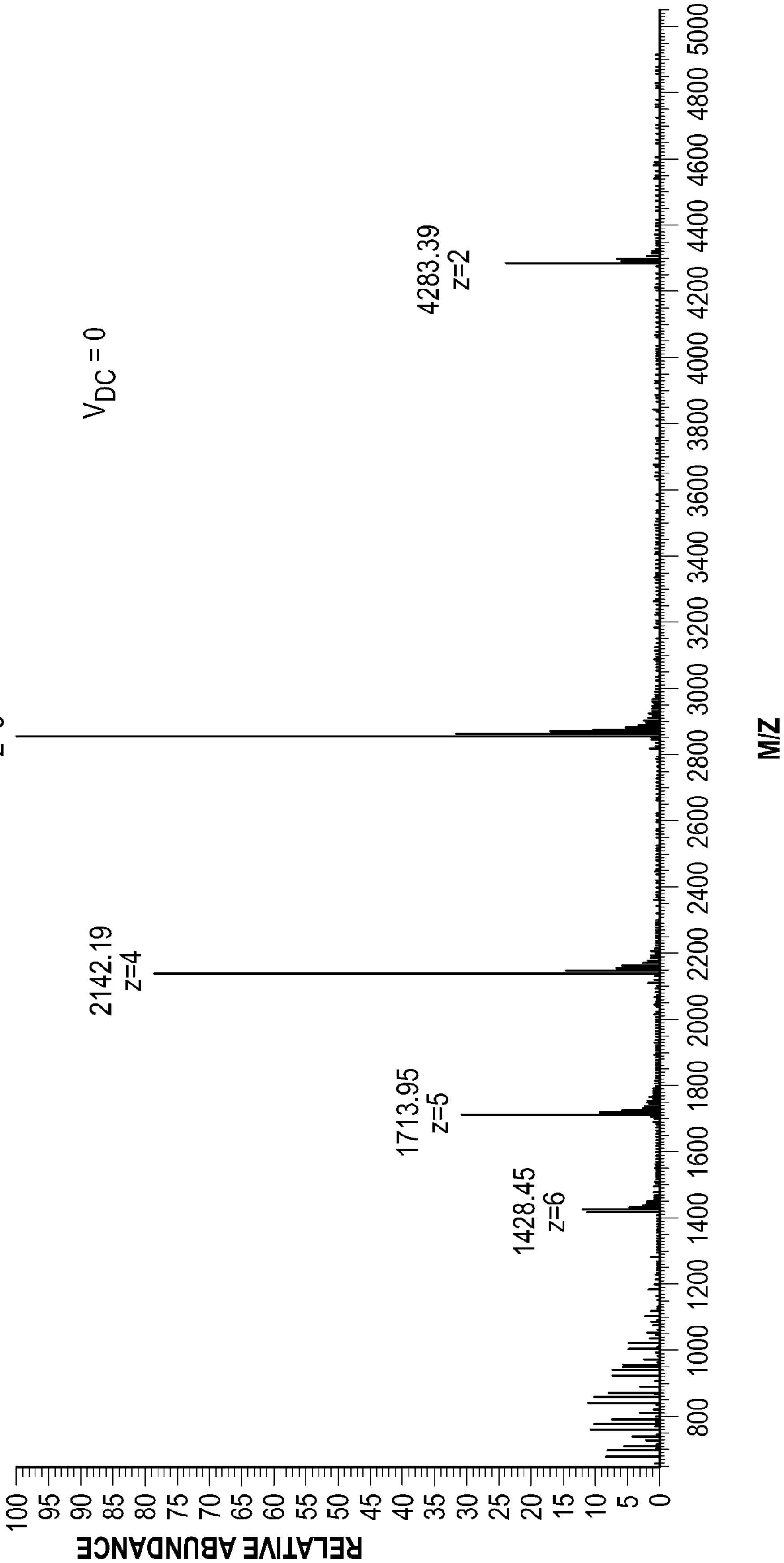

2+
3+
4+
5+
6+
7+
8+
9+
10+
11+
12+
FRACTION OF ALL CHARGE STATES
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
VOLTAGE
100
200
300
400
500
600
700
800
900
1000
200
$V_{DC1}$
$V_{DC2}$
$V_{DC3}$
$V_{DC4}$

*FIG. 5*



$V_{DC} = 0$
4283.39
z=2
2855.58
z=3
2142.19
z=4
1713.95
z=5
1428.45
z=6
RELATIVE ABUNDANCE
100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000 4200 4400 4600 4800 5000
M/Z


*FIG. 6*

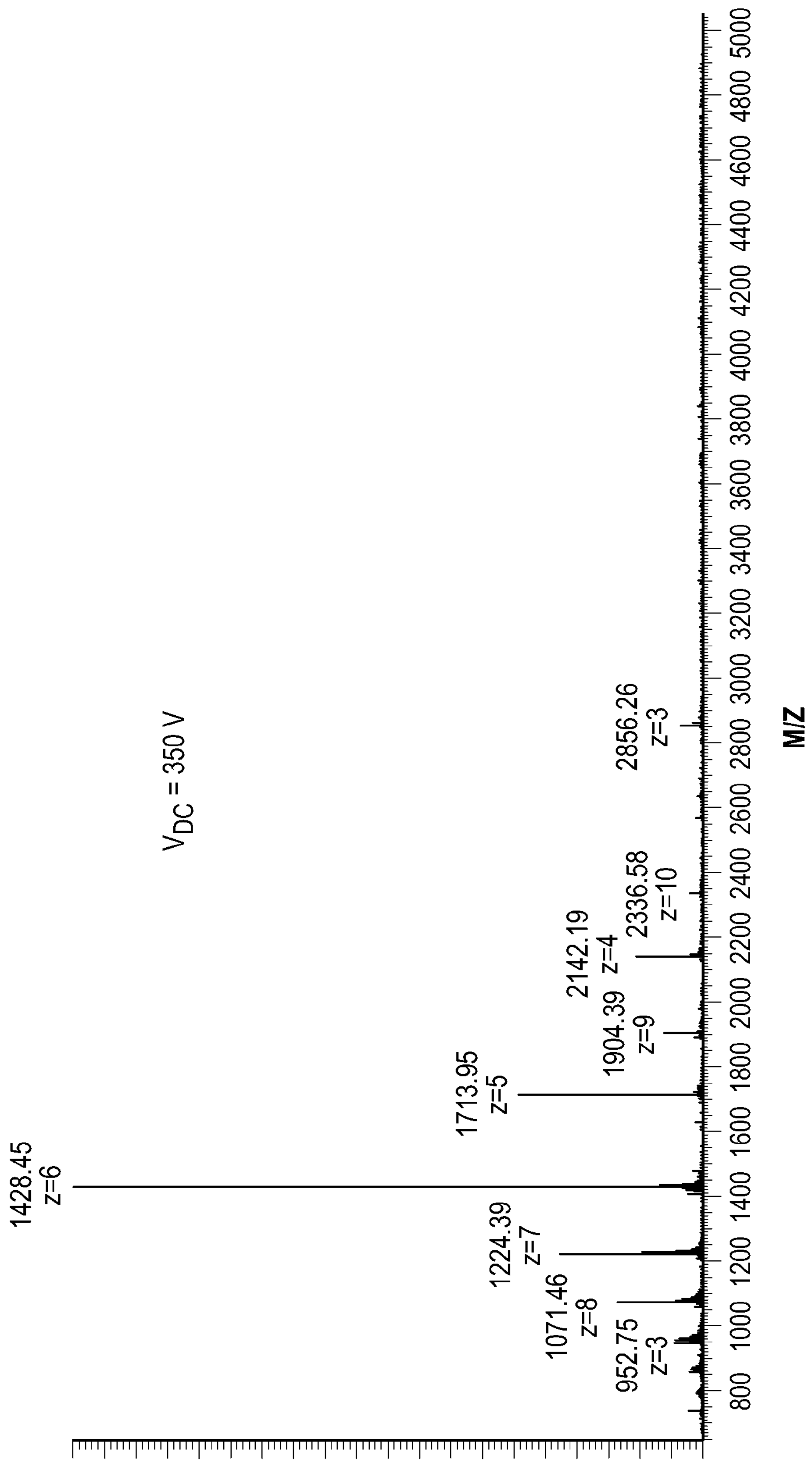

V$_{DC}$ = 350 V
1428.45
z=6
1224.39
z=7
1071.46
z=8
952.75
z=3
1713.95
z=5
1904.39
z=9
2142.19
z=4
2336.58
z=10
2856.26
z=3
RELATIVE ABUNDANCE
M/Z
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000 4200 4400 4600 4800 5000

*FIG. 7*

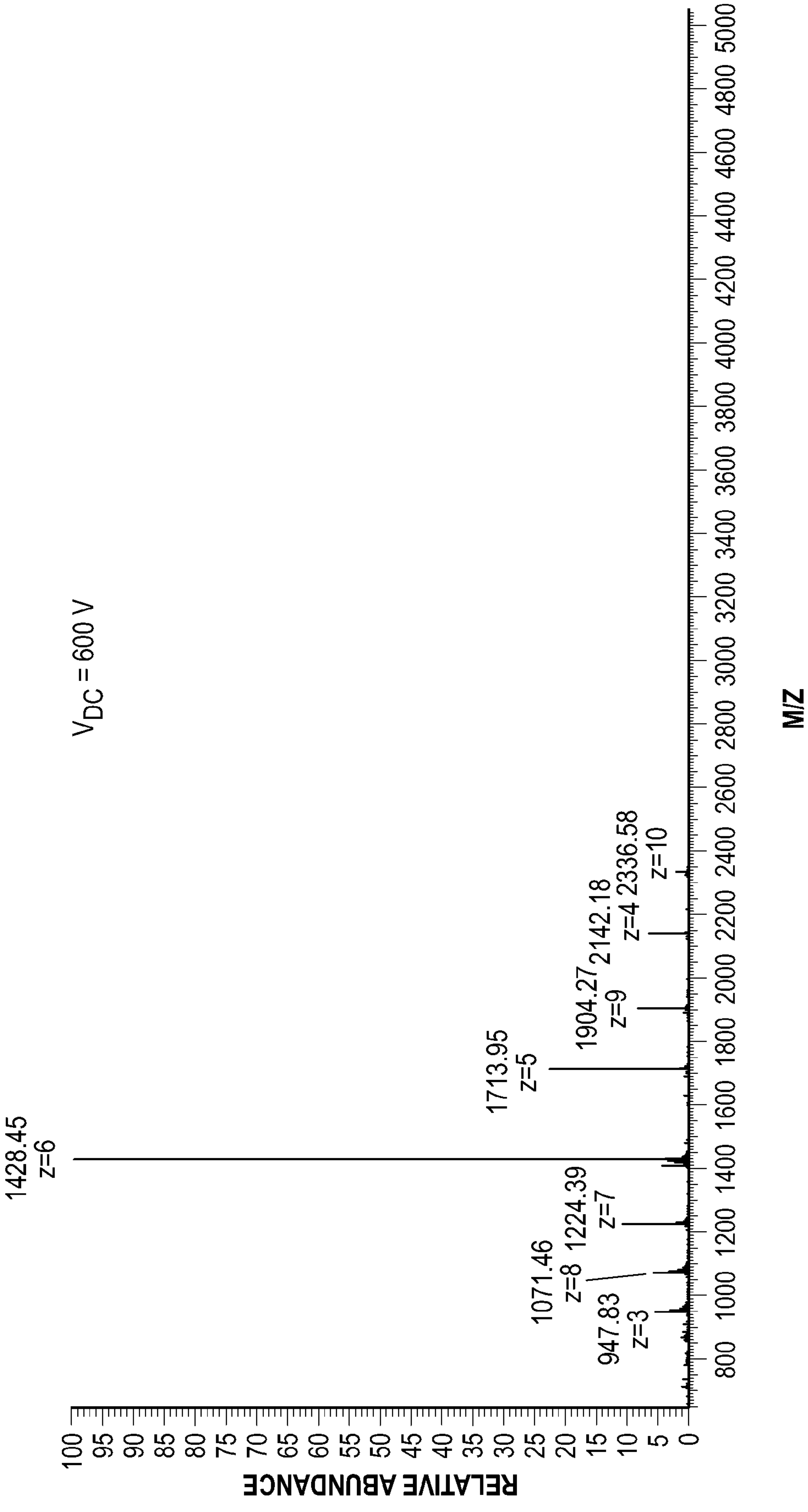

$V_{DC}$ = 600 V
1428.45
z=6
1713.95
z=5
1904.27
z=9
2142.18
z=4
2336.58
z=10
1224.39
z=7
1071.46
z=8
947.83
z=3
RELATIVE ABUNDANCE
100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800 3000 3200 3400 3600 3800 4000 4200 4400 4600 4800 5000
M/Z

*FIG. 8*

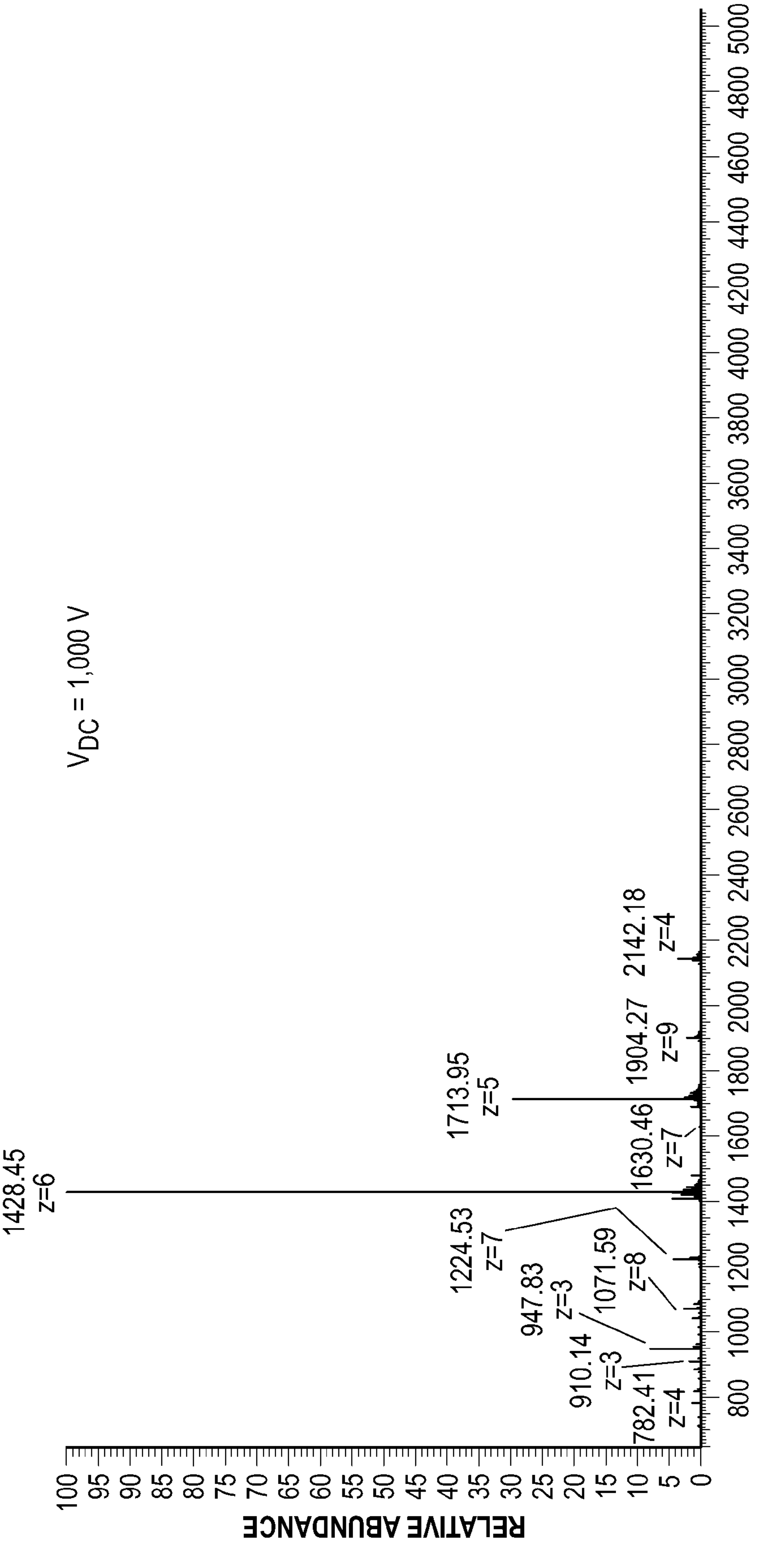

$V_{DC}$ = 1,000 V
RELATIVE ABUNDANCE
M/Z
1428.45
z=6
1713.95
z=5
1224.53
z=7
1630.46
z=7
1904.27
z=9
2142.18
z=4
947.83
z=3
1071.59
z=8
910.14
z=3
782.41
z=4
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
4000
4200
4400
4600
4800
5000

*FIG. 9*

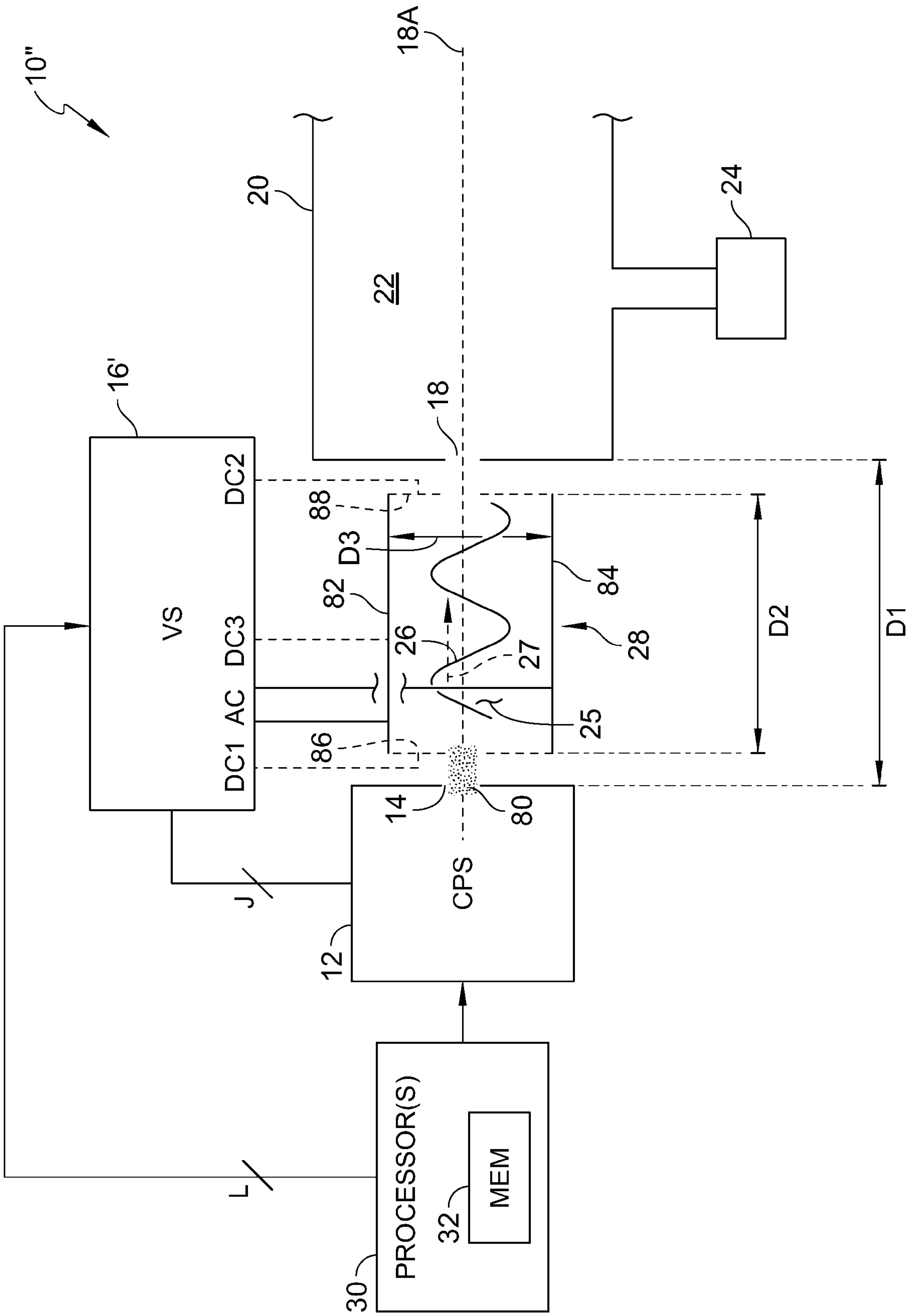

10"
18A
20
22
24
16'
VS
DC2
DC3
AC
DC1
88
D3
82
84
26
27
28
D2
D1
25
86
14
80
CPS
J
12
L
PROCESSOR(S)
MEM
32
30

FIG. 10

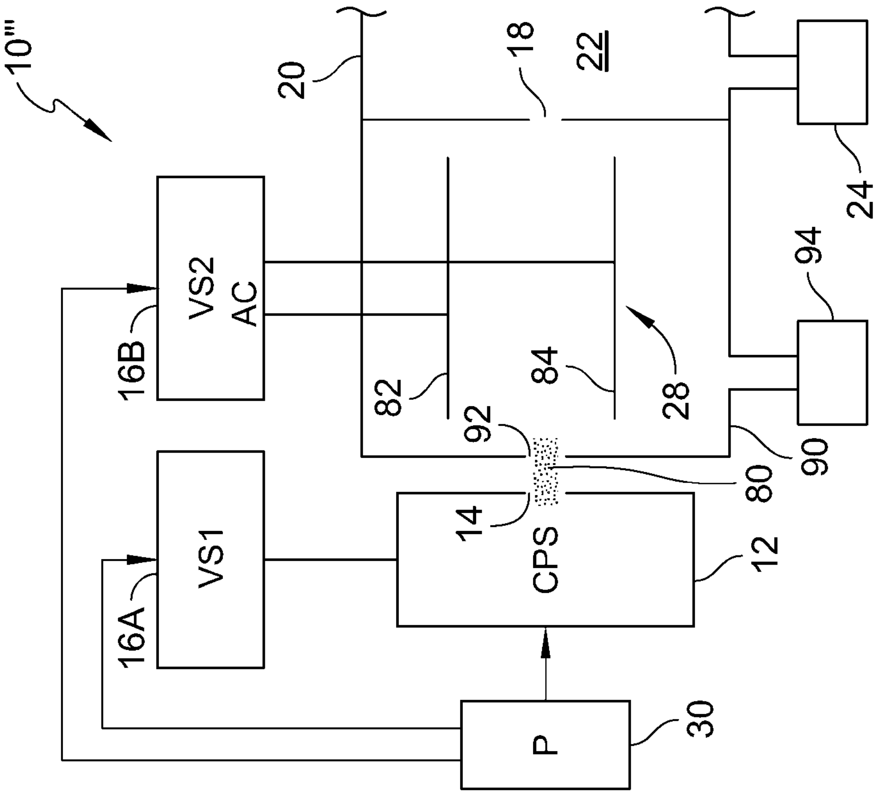



*FIG. 11*

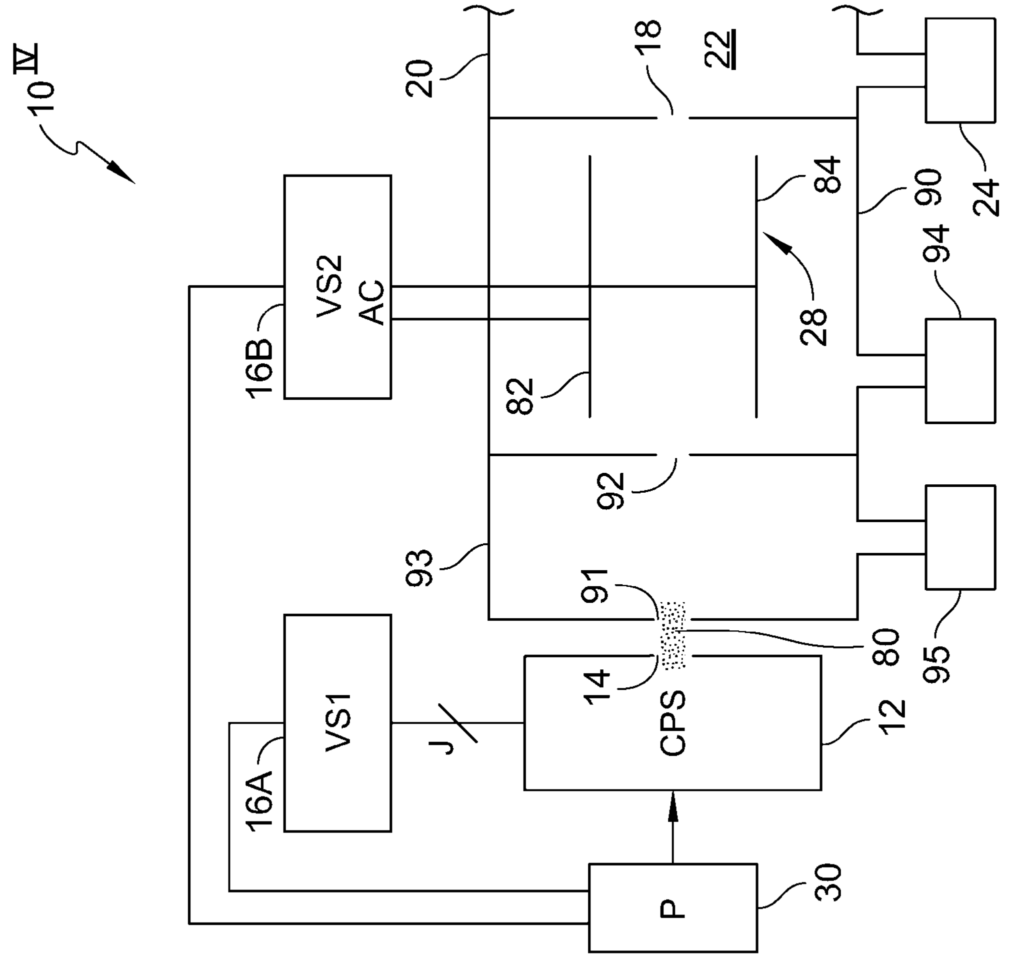

10IV
VS2
AC
16B
VS1
16A
J
CPS
P
12
14
30
20
18
22
84
90
24
28
82
94
92
93
91
80
95

FIG. 12

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 24/49386

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. B05B 5/025 (2024.01)
ADD. B05D 1/06 (2024.01)

CPC - INV. B05B 5/001, B05B 5/025

ADD. B05B 5/007, B05D 1/06, G01N 30/724

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2016/0187297 A1 (SHARP et al.) 30 June 2016 (30.06.2016) Fig 1-3, abstract, para [0014], [0024], [0050] | 1-6, 14-16 |
| A | US 2009/0078800 A1 (NAKASONE et al.) 26 March 2009 (26.03.2009) abstract | 1-6, 14-16 |
| A | US 2021/0315278 A1 (NICOVENTURES TRADING LIMITED) 14 October 2021 (14.10.2021) abstract | 1-6, 14-16 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 07 November 2024 | DEC 10 2024 |

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Kari Rodriquez<br>Telephone No. PCT Helpdesk: 571-272-4300 |

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 24/49386

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-13, 17-27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.