(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 211 645**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: **86306004.2**

(22) Date of filing: **04.08.86**

(51) Int. Cl.⁴: **G 01 N 27/62**
H 01 J 49/04

(30) Priority: **21.08.85 US 767819**
21.08.85 US 767820

(43) Date of publication of application:
25.02.87 Bulletin 87/9

(84) Designated Contracting States:
DE FR GB

(71) Applicant: **SPECTROS LIMITED**
Barton Dock Road
Urmston Manchester M31 2LD(GB)

(72) Inventor: **Caprioli, Richard M.**
5637 Lucerne
Bellaire Texas 77401(US)

(72) Inventor: **Cottrell, John S.**
33 Bamford Road
Disbury Manchester M20 8QP(GB)

(74) Representative: **Taylor, Derek George et al,**
MATHISEN, MACARA & CO. The Coach House 6-8
Swakeleys Road
Ickenham Uxbridge UB10 8BZ(GB)

(54) **Apparatus and methods for use in the mass analysis of chemical samples.**

(57) A probe for supporting a sample in an ion source of a mass spectrometer is arranged to receive a continuous supply of liquid containing a sample to be analysed, which sample may, or may not, change with time. The probe can comprise a target formed by a copper probe tip 18 and the liquid sample can pass, for example, through a fine bore 18a of the tip, on to the end surface of the tip where it is held as a droplet by surface tension. In order to replenish the droplet surface, liquid sample can be supplied, for example via capillary tubing 14 from a syringe 11, to the inlet end of the bore in the probe tip.

EP 0 211 645 A2

Croydon Printing Company Ltd.

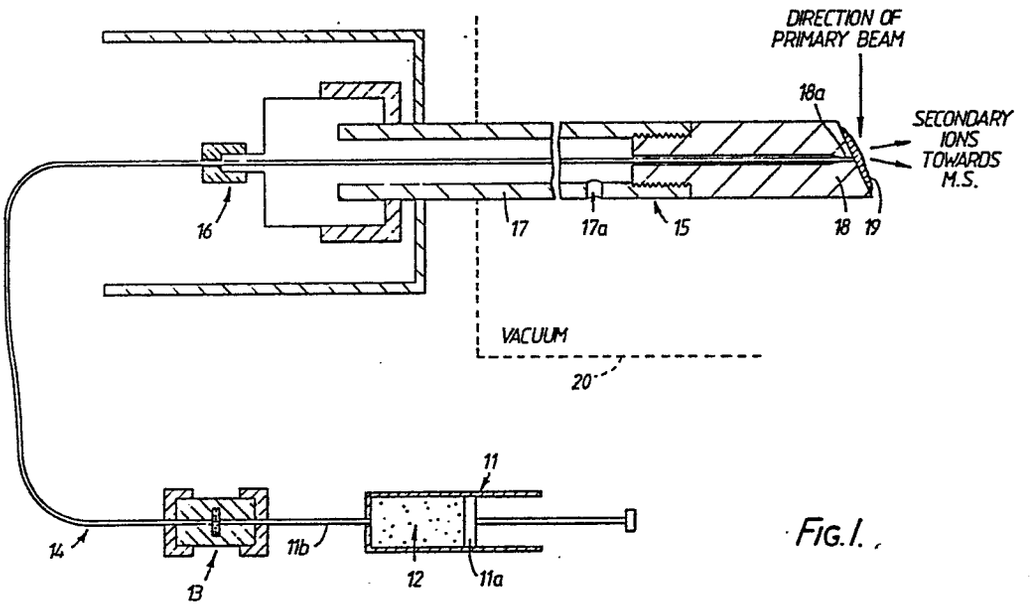
DIRECTION OF
PRIMARY BEAM
18a
SECONDARY
IONS
TOWARDS
M.S.
16
17
17a
15
18
19
VACUUM
20
11
14
11b
12
11a
13

FIG. 1.

APPARATUS AND METHODS FOR USE IN THE MASS ANALYSIS OF CHEMICAL SAMPLES

This invention relates to mass spectrometry apparatus for use in the continuous analysis of a chemical sample, and methods of using such apparatus.

Methods of using mass spectrometers for the analysis of samples which do not change with time are well known, but in the use of mass spectrometers for analysis of the time profile of a chemical reaction, the problem arises of locating the sample in an ion source at the moment at which the reaction products are to be analysed.

It has been suggested (see Anal Chem 1985,57,1153-55) that the time profile of a chemical reaction can be monitored by passing aqueous samples through semipermeable silicon capillary tubing of which a loop is sealed within a high vacuum system, the inlet and outlet ends of the tubing being outside the vacuum system. Samples passing through the tubing wall enter an area which is connected by ducting to an ion source of a triple quadrupole mass spectrometer. However, this approach is only applicable to the analysis of volatile samples.

According to one aspect of the present invention there is provided mass spectrometry apparatus for use in the continuous analysis of a sample of which the composition may, or may not, change with time, which comprises a high vacuum system, means for depositing a supply of the sample on a surface located within the high vacuum system, means for ionising the deposited sample in situ on the surface and means for mass analysing the ions so produced.

The present invention further provides apparatus for use, in association with a mass spectrometer including a high vacuum analyser system and a sputtering ion source, in the continuous analysis of a sample of which the composition may, or may not, change with time, which apparatus comprises a target carrier adapted to be connected to the high vacuum analyser system so that a surface of the target carrier subject to the high vacuum within the system can form a target for said ion source, and means effective during the operation of the apparatus, to conduct a flow of liquid in which the sample is carried from a location, which is at a high pressure relative to that within said system, to the said surface which is subject to the high vacuum.

According to another aspect the present invention provides a method for the continuous analysis of a sample of which the composition may or may not change with time which comprises continuously depositing a supply of the sample on a surface located within a high vacuum system, ionising the deposited sample in situ on the surface and mass analysing the ions so produced.

The present invention also provides a method for the continuous analysis of a sample of a biopolymer, such for example as a protein, which comprises reacting the biopolymer sample with a substance which sequentially removes terminal units of the biopolymer, depositing a supply of the reaction products continuously on the target surface of a sputtering ion source, causing ionisation of the deposited reaction products and mass analysing the ions so produced to permit identification of the terminal units sequentially removed from the biopolymer by determining the reduction with time of the molecular mass of the biopolymer as the terminal units are removed.

In particular the present invention provides a probe for insertion into a mass spectrometer which, in operation, permits a continuous replenishment of the

sample at the target for irradiation. The mass spectrometer source in which the probe is located ionises the sample by F.A.B. (Fast Atom Bombardment), or any other sputtering technique.

The invention will now be more particularly described by way of example only, with reference to the accompanying drawing in which:-

Figure 1 is a diagrammatic representation of one embodiment of apparatus in accordance with the present invention;
Figure 2 is a diagrammatic section through a modified form of probe;
Figure 3 is a diagrammatic section through an alternative form of probe;
Figure 4 is a diagrammatic section through the probe tip of yet another form of probe, and
Figure 5 is a diagrammatic section through yet another form of probe.

The apparatus illustrated in Figure 1 of the drawing is designed to allow a sample under investigation to be introduced into the high vacuum system of a mass spectrometer.

The apparatus comprises a gas-tight syringe 11 of suitable capacity (eg 50 or 100 microlitres). This syringe is mounted on a mechanical actuator known as a "syringe pump" which moves a plunger 11a of the syringe at constant rate so as to provide a known flow of mixture 12 out of the needle 11b of the syringe The mixture 12 would be typically 90 microlitres degassed water, 10 microlitres degassed glycerol, the sample under investigation (e.g. a peptide at a concentration of 1 microgram per microlitre), an enzyme mixture, buffer salts and other ingredients dependent on the nature of the experiment. Coupling means 13 is used to connect the syringe needle to a length of fused quartz capillary tubing 14. This coupling means may conveniently include an in-line filter to remove particulate matter from the liquid flow which might otherwise block the capillary tubing 14. Capillary tubing 14 is typically a 1 metre length of 25 micrometre internal diameter fused quartz. The length and diameter are chosen such that only a few atmospheres of pressure are required to produce the desired flow rate. Capillary tubing 14 enters a probe assembly 15 through coupling means 16 which provides a vacuum tight seal. The probe assembly includes a hollow shaft 17 through which the capillary tubing 14 passes into a probe tip 18 through which a capillary

bore 18a extends. The inner end of the bore of the probe tip 18 is a close fit to the capillary tubing 14 so as to provide good thermal contact between the probe tip 18 and the end of the capillary tubing 14. Preferably the probe tip is made of copper for good heat transfer. A vent 17a is provided in shaft 17 for efficient evacuation of the hollow probe shaft. In operation, a bead 19 of glycerol solution forms on the tip 18 at the outlet end of the bore 18a as a result of expulsion of solution through the tip 18, the liquid bead being retained by surface tension on the probe tip surface surrounding the bore outlet. The shape and angle of inclination of the end surface of probe tip 18 will depend on the geometry of the mass spectrometer ion source.

The probe tip is sealed into the high vacuum chamber 20 (shown by a dotted line) of the ion source of a mass spectrometer, with the outlet end of the probe tip bore located at a position on the path of a primary beam of radiation.

The principle of operation will now be described. Enzymatic reactions can only proceed in aqueous solution. On exposure to a vacuum, the water content of any solution will evaporate rapidly and the

reaction will cease. This apparatus provides a means of introducing a continuous flow of reaction mixture into a mass spectrometer ion source without exposing the bulk of the mixture to the vacuum.

When the reaction mixture is pumped through a suitable capillary at a flow rate of about 1 microlitre per minute, evaporation will not take place until the fluid emerges from the tip of the capillary. At this point, the water content of the mixture will evaporate rapidly, while the less volatile glycerol content will flow onto the probe tip end surface. Only a fraction of the glycerol will evaporate during the experiment; the area of the end surface of the probe tip (e.g. 30 square millimetres) is sufficient to support this volume of glycerol without it forming an unwieldy droplet. Approximately 54 microlitres of water will evaporate into the source vacuum each hour. A typical mass spectrometer pumping system can cope with this flow rate and still maintain an adequate source vacuum.

Heat must be applied to the capillary tip if the continuous evaporation of water is not to result in the mixture freezing. To facilitate heat transfer, the probe tip is in good thermal contact with the

capillary tubing. In our apparatus, the energy incident on the probe tip from the primary particle beam is sufficient to maintain it at room temperature. Under other circumstances some heating means, such as an electrical resistance heater, would be required.

A further advantage of using a fine quartz capillary is that the resistance of a 1 metre length is sufficient to prevent voltage breakdown between the probe tip and ground. In a magnetic mass spectrometer the probe tip may be at a potential of 10,000 V.

It may be advantageous to have control over the rate of chemical reaction within mixture 12. For example, the reaction could be inhibited during the loading of the syringe and during the insertion of the probe into the mass spectrometer source. Such control may be obtained through temperature regulation of mixture 12. Reduction of the temperature to 0°C will inhibit the reaction whilst warming to body temperature will accelerate the reaction. Temperature regulation of the syringe and its contents could be provided by a water jacket. Temperature regulation of the capillary will not normally be necessary, although thermal insulation by means of heat insulating sleeving would be desirable.

A typical experimental procedure would be as follows: Syringe 11 is filled with a degassed solution of 90 microlitres water, 10 microlitres glycerol, Substance-P (a polypeptide) and a mixture of carboxypeptidase Y and carboxypeptidase B. The relative concentrations of the enzymes are such as to give complete hydrolysis of the polypeptide over the duration of the experiment (typically a few minutes per amino acid residue). The syringe is then coupled to the probe system as shown in the drawing.

The probe is introduced through a vacuum lock into a standard FAB source. The syringe pump is set to a flow rate of about 1 microlitre per minute. A beam of primary particles or radiation is allowed to impinge upon the surface of the reaction mixture eluting on to the probe tip end surface. This primary beam would typically be xenon atoms, but could equally well be caesium ions, fission fragments, or photons. The primary beam causes ions to be sputtered from the surface of the reaction mixure. These ions are then drawn into a mass spectrometer and mass analysed.

Observation of the mass spectrum of the reaction mixture will reveal the following features:-

Initially, there will be a strong peak corresponding to the intact polypeptide molecule. If the mass spectrometer is transmitting positive ions this will be the protonated molecular ion $(M+H)^+$. In the case of Substance-P (H-Arginine-Proline-Lysine-Proline-Glutamine-Glutamine-Phenylalanine-Phenylalanine-Glycine-Leucine-Methionine-OH) the protonated molecular ion is observed at m/z 1348. As the polypeptide is digested by the enzyme mixture, amino acid residues are sequentially removed from the C terminus of the chain, and we observe the appearance of new molecular ions corresponding to the loss of Met (yielding m/z 1217), loss of Leu (yielding m/z 1104). Thus the mass difference between consecutive molecular ions identifies the amino acid residue removed from the chain, so yielding the amino acid sequence of the polypeptide. The only ambiguity in the sequence information provided by this technique is failure to distinguish between residues of the same molecular weight. Amongst the common amino acids there are only two examples of this: Glutamine and Lysine (both m/z 128) and the isomers Leucine and isoLeucine (both m/z 113).

An advantage of this technique is that the molecular ion intensities are obtained as a function of time.

Some molecular ion peaks will be of relatively low intensity, possibly because the ion is produced by a cleavage which occurs particulary slowly resulting in a low instantaneous concentration of that species. Observation of the time dependant behaviour of the "parent" and "daughter" molecular ions will allow the time dependance of the "missing" molecular ion to be predicted. Since there will be only one or two possible mass values for the "missing" ion, this information will enable extremely weak molecular ions to be distinguished from interfering peaks which do not show the expected time dependence.

It will be appreciated that the application of this technique is not restricted to the C-terminus sequencing of peptides and proteins. Use of aminopeptidase enzymes permits peptides to be sequenced from the N-terminus. Alternatively, polysaccharides, oligonucleotides and other biopolymers may be sequenced using the appropriate reaction mixture.

The cell would also be ideal for the observation and measurement of enzyme kinetics and any experiment in which observation time would be limited by evaporation of a volatile solvent or matrix.

A modification of the apparatus for performing the invention is illustrated in Figure 2. The probe illustrated in Figure 2 comprises at least three sections, a first or terminal section 30 which forms a target carrier, the section being of tubular shape and screwthreaded to a second tubular section 31 to grip therebetween the rim of a semi-permeable membrane 32. The cylindrical space within the tube section 30 forms a reaction cell 33 the axially-inner boundary of which is defined by the semi-permeable membrane 32. At the outer end of the cell, a high transparency stainless steel mesh 34 can be located to assist in defining a physical boundary of liquid located in the cell.

A third tubular section 35 forms, or is mounted on, the main part of the probe shaft 41, and is screwthreaded to the axially-inner end of section 31 to grip therebetween the rim of a flexible impermeable membrane 36. Membranes 32 and 36 define between them a reservoir 37 within section 31. The axially-outer end portion of section 35 is hollow to form a chamber 38 which is closed at its outer end by the impermeable membrane 36 and can communicate with a pressure source via an equilibration vent 40 in the wall of section 35.

The inner end of the probe shaft 41 can support an ultrasonic transducer 42 for a purpose to be described below. The probe shaft, in use, can be introduced into the mass spectrometry source through a vacuum lock, without venting the source. The tubular sections 30,31 and 35 thus form a casing which separates the high vacuum within the ion source from the relatively high ambient pressure, yet permits liquid to be introduced into the vacuum space through a restricted path formed by the semipermeable membrane 32, or otherwise as described below.

An example of the use of the probe will now be considered. The reaction cell 33 contains a solution of 50:50 V/V glycerol and water, together with the sample under investigation (e.g. a peptide at a concentration of 5 microgrammes per microlitre), an enzyme mixture, buffer salts and other ingredients dependent on the nature of the experiment. The volume of the total mixture will be typically 20 microlitres. The purpose of the reaction, as in the case of the first embodiment, is to permit enzymatic degredation of the sample to take place and to allow the reaction products to be brought to the target of the probe where they will be ionised in situ and the ions to be mass analysed so that a time profile is produced of

the reaction between the enzyme and the sample.

Since the diameter and length of the reaction cell will be typically three millimetres, the droplet of liquid within the cell will be retained by surface tension.

Reservoir 37 contains either pure water, or a solution of glycerol in water. The semipermeable membrane 34, which can be polymeric, conveniently has an effective molecular weight cut off of about 1000 amu (e.g. Millipore "Pellicon" ultrafiltration disc or thin cellulose sheet). Alternatively it could be a sintered porous material, a perforated plate, or a gauze or mesh. Its essential property is that it is water permeable. The purpose of the impermeable membrane 36 is to permit volume changes to occur in the reservoir without alteration in the pressure. Membrane 36 could therefore be replaced by a sliding gas-tight plunger. The vent 40 maintains pressure equilibrium during pump down and venting of the probe.

The principle of operation will now be described. Since the vapour pressure of water is extremely high relative to that of glycerol, on exposure to the high vacuum, water is lost from the surface of the mixture

in reaction cell 33 at a much greater rate than glycerol. Shortly after introduction into the high vacuum source of the mass spectromer, a water concentration gradient will be created, the water content of the mixture in cell 33 being very low at the surface and very high at the interface with membrane 32. Under steady state conditions a diffusion limited flow of water will take place from the membrane 32 to the surface of the mixture in cell 33. The magnitude of the concentration gradient, and hence the rate of diffusion of water towards the surface, will depend on the distance between the membrane interface at the inner end of cell 33 and the vacuum interface at the outer end of cell 33. The enzymatic hydrolysis of the sample in cell 33 can only proceed in the presence of water. The purpose of reservoir 37 is to replenish the water content of the reacting mixture in cell 33 by diffusion through membrane 32. In order to maintain the volume of mixture constant over an extended time period, it may be found necessary to include a percentage of glycerol in reservoir 37.

This reaction cell relies on the continuous diffusion of reaction products towards the surface of mixture in cell 33, where ionisation takes place, and the

complementary diffusion of material from the surface back into the central regions of the cell where there is sufficient water for further cleavage to occur. The rate of transport of material between these regions may be accelerated by ultrasonic agitation caused by the transducer 42 embedded in, or adjacent to, probe shaft 41.

It may be required to have independent control over the pressure in reservoir 37. This could be achieved by omitting the equilibration vent 40 and coupling chamber 38 to an external pressure control apparatus by means of a tube passing through the probe shaft 41. The pressure of gas or fluid in chamber 38 would be transmitted to reservoir 37 via flexible membrane 36. Alternatively, the flexible membrane 36 could be replaced by a rigid wall into which a tube is sealed connecting reservoir 37 directly with an external pressure control apparatus. In this case, the pressure transmitting fluid could be of the same composition as the water/glycerol solution in reservoir 37. Provision of a second connecting tube between reservoir 37 and the external pressure control apparatus would enable the water/glycerol solution to be circulated between the external apparatus and reservoir 37. By this means, the temperature of the

probe tip could be regulated according to the temperature of the solution. This would provide additional control over the rate of reaction, since the enzymes generally function most efficiently at about 37°C.

A typical experimental procedure would be as follows: During assembly of the probe, reservoir 37 is filled with a degassed solution of 90% water, 10% glycerol (by volume). Reaction cell 33 is filled with a degassed solution of 10 microlitres water, 10 microlitres glycerol, Substance-P (a polypeptide) and a mixture of carboxypeptidase Y and carboxypeptidase B. The relative concentrations of the enzymes are such as to give complete hydrolysis of the polypeptide over the duration of the experiment (typically a few minutes per amino acid residue).

The probe is introduced through a vacuum lock into a standard FAB source. A short period is allowed for the mixture to equilibrate under vacuum conditions. A beam of primary particles or radiation is allowed to impinge upon the surface of the reaction mixture. This primary beam, as before, would typically be xenon atoms, but could equally well be caesium ions, fission fragments, photons, etc., etc. The primary beam

causes ions to be sputtered from the surface of the reaction mixure. These ions are then drawn into a mass spectrometer and mass analysed.

Observation of the mass spectrum of the reaction mixture will reveal the same features as were described above in relation to the first embodiment of the invention.

As in the case of the first embodiment, the cell would also be ideal for the observation and measurement of enzyme kinetics and any experiment in which observation time would be limited by evaporation of a volatile solvent or matrix.

A modification of the probe which enables a continuous flow of liquid sample to be analysed, is illustrated in Figure 3.

As shown, the probe tip 45 comprises a solid cylindrical tip member 46 having an end surface 47 inclined to the axis of the probe and containing a recess, forming a reservoir 48, which is closed on its outer side by a semi-permeable membrane 50. This membrane can be made of any of the materials described in relation to membrane 32. The membrane 50 is held

in position on the end of the tip member by an annular cap 51. Capillary passages 52 extend parallel to the axis of the probe, through the length of tip member into the reservoir 48. The tip member is screwthreaded to a tubular shaft 53 through which capillary tubes 54, for example of quartz, extend and are sealed to the rearward ends of the capillary passages.

A further modification of the probe, again enabling a continuous flow of liquid sample to be analysed, is illustrated in Figure 4.

As shown, the probe tip 55 comprise a tip member 56 which contains a central capillary passage 57 leading to a cup portion 58 at the forward extremity of the tip member. The lip 60 of the cup portion has a sharp edge and the cup portion is surrounded by an annular overflow channel 61.

In this case, a slow continuous delivery of a liquid sample with a high surface tension enables a dome-shaped droplet to form in the cup. If the delivery rate of the sample is maintained slightly greater than the evaporation rate of the liquid component of the sample, a continuous overflow will occur to maintain a

constant dome shape of the droplet in the cup.

Another embodiment of the invention which permits a continuous flow of liquid sample to be analysed, is illustrated in Figure 5.

The probe tip 65, shown in Figure 5, comprises a tubular tip member 66 closed at its outer end by an end wall 67 containing two apertures 68,69 in which the ends of supply and return pipes 71,72 are sealed. A cover plate 73, for example of stainless steel, is fitted to the end wall and sealed thereto around its periphery to form therebetween a chamber 74 into which the supply pipe 71 discharges and from which the return pipe 72 discharges. In this way a continuous flow of sample can pass through the chamber.

At the centre of the cover plate 73, is a restricted outlet orifice 75 which leads into a cup 76 for containing liquid sample. Surrounding the cup, and flush with the lip 77 of the cup, is a porous ceramic mass 78 into which liquid sample which overflows the lip of the cup can be absorbed.

The hollow interior of the tubular tip member also contains a liquid supply pipe 80 through which water

or other suitable liquid can be caused to flow over the rearward surface of the end wall and thereby control the temperature of the end wall and the liquid sample within the reservoir.

The tip member 66 is mounted on a tubular probe shaft 81 through which extend the sample supply and return pipes 71,72 as well as the temperature-control liquid supply pipe 80.

In operation of this embodiment, the flow of liquid sample, which can be a reaction mixture, through the orifice is at a rate slightly greater than the rate of evaporation, and the excess is absorbed by the porous ceramic mass. A convenient flow rate would be about one microlitre per minute.

The shape and angle of inclination of the end surface of the probe tip in each of the embodiments described above will depend on the geometry of the mass spectrometer ion source.

CLAIMS

1. Mass spectrometry apparatus for use in the continuous analysis of a sample of which the composition may, or may not, change with time, which comprises a high vacuum system, means for depositing a supply of the sample on a surface located within the high vacuum system, means for ionising the deposited sample in situ on the surface and means for mass analysing the ions so produced.

2. Apparatus for use, in association with a mass spectrometer including a high vacuum analyser system and a sputtering ion source, in the continuous analysis of a sample of which the composition may, or may not, change with time, which apparatus comprises a target carrier adapted to be connected to the high vacuum analyser system so that a surface of the target carrier subject to the high vacuum within the system can form a target for said ion source, and means effective, during the operation of the apparatus, to conduct a flow of liquid in which the sample is carried from a location, which is at a high pressure relative to that within said system, to the said surface which is subject to the high vacuum.

3. Apparatus according to claim 1 or claim 2 wherein the means for conducting a flow of liquid comprises a vessel for containing the sample and the liquid in which the sample is dissolved or otherwise carried, together with any substance with which the sample may be required to react, and a fluid-flow ducting for conducting a flow of said liquid containing said sample or the reaction products of said sample and said substance from the vessel to the said surface.

4. Apparatus according to claim 2 or claim 3 wherein the target carrier is formed by the tip of a probe having an external surface which surrounds an outlet orifice and forms the target surface, and said ducting, which is formed by capillary tubing, opening via said orifice on to said target surface so that the liquid containing the sample or said reaction products can pass through the orifice on to said target surface.

5. Apparatus according to claim 4 wherein the probe tip is made of copper.

6. Apparatus according to claim 4 or claim 5 wherein a syringe containing a supply of said liquid is connected to an inlet end of said capillary tubing.

7. Apparatus according to claim 2 or claim 3 wherein the target carrier includes a semi-permeable membrane capable of withstanding the pressure difference between said high pressure and said high vacuum, said semi-permeable membrane having said target surface on the high vacuum side thereof and said ducting opening on to said target carrier on the high pressure side thereof so that the liquid together with the sample or said reaction products carried thereby can pass through the membrane on to said target surface.

8. Apparatus according to claim 7 wherein the membrane is a glass frit or other sintered material having an electrically-conductive coating of gold or other electrically conductive material on its target surface.

9. Apparatus according to claim 2 or claim 3 wherein a part of said target carrier is shaped to form a receptacle for a droplet of liquid and the means for conducting a flow of liquid open into said receptacle, so that the droplet can be replenished as liquid evaporates from its surface.

10. Apparatus according to claim 9 wherein a membrane through which liquid can permeate is interposed in the path of liquid flowing to said receptacle.

11. Apparatus according to claim 9 wherein a membrane through which liquid can permeate extends across the mouth of said receptacle.

12. Apparatus according to claim 9 wherein said receptacle has a sharp edged lip and an overflow channel surrounds said lip.

13. Apparatus according to claim 9, wherein said receptacle is formed with a lip, and a mass of porous material surrounds said lip and extends substantially flush therewith, said material being arranged to absorb liquid overflowing the lip and thereby influence the shape of the liquid meniscus at the mouth of the receptacle.

14. Apparatus according to claim 9 wherein the means for conducting a flow of liquid comprises an inlet pipe, and a return pipe communicates with said inlet pipe at or adjacent to said receptacle to conduct liquid away from the receptacle.

15. Apparatus according to claim 3 wherein the target carrier includes a solid target member having thereon said target surface, and a casing supporting the target member and capable of withstanding the pressure difference between said high pressure and said high vacuum, said ducting extending through and being sealed at said casing and opening adjacent said target surface so as to discharge liquid on to said target surface.

16. A method for the continuous analysis of a sample of which the composition may or may not change with time which comprises continuously depositing a supply of the sample on a surface located within a high vacuum system, ionising the deposited sample in situ on the surface and mass analysing the ions so produced.

17. A method for the continuous analysis of a sample of a biopolymer, such for example as a protein, which comprises reacting the biopolymer sample with a substance which sequentially removes terminal units of the biopolymer, depositing a supply of the reaction products continuously on the target surface of a sputtering ion source, causing ionisation of the deposited reaction products and mass analysing the

ions so produced to permit identification of the terminal units sequentially removed from the biopolymer by determining the reduction with time of the molecular mass of the biopolymer as the terminal units are removed.

18. A method according to claim 16 or claim 17 which includes ionising the deposited sample by causing a beam of particles or of radiation to impinge thereon.

19. A method according to claim 17 or claim 18 which includes dissolving the sample in a mixture of water and glycerol or other fluid substance less volatile than water so that after the water content of the deposited supply has evaporated, the material of the sample or its residue will be contained in a globule of said fluid substance.

0211645

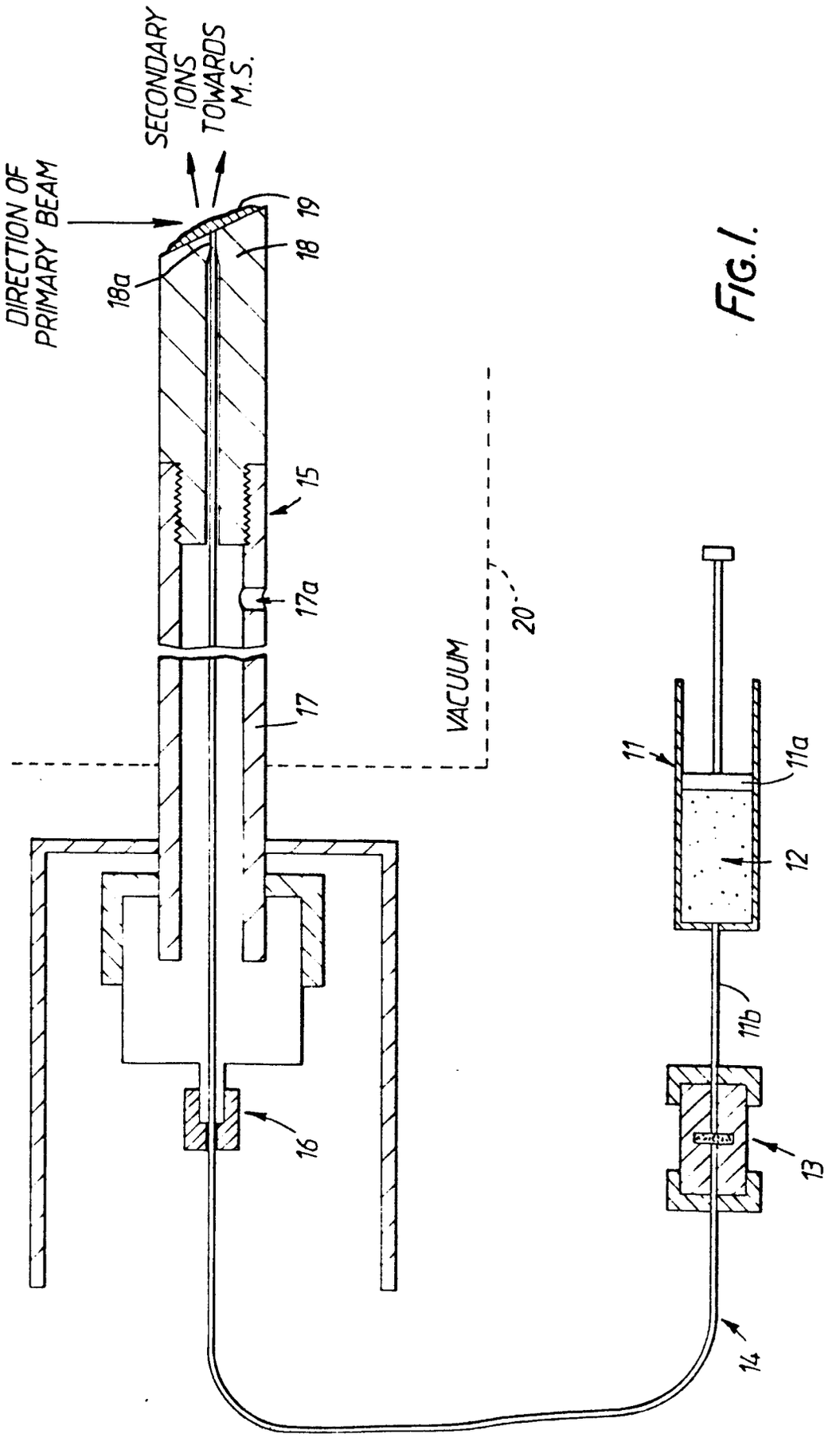

FIG. 1.

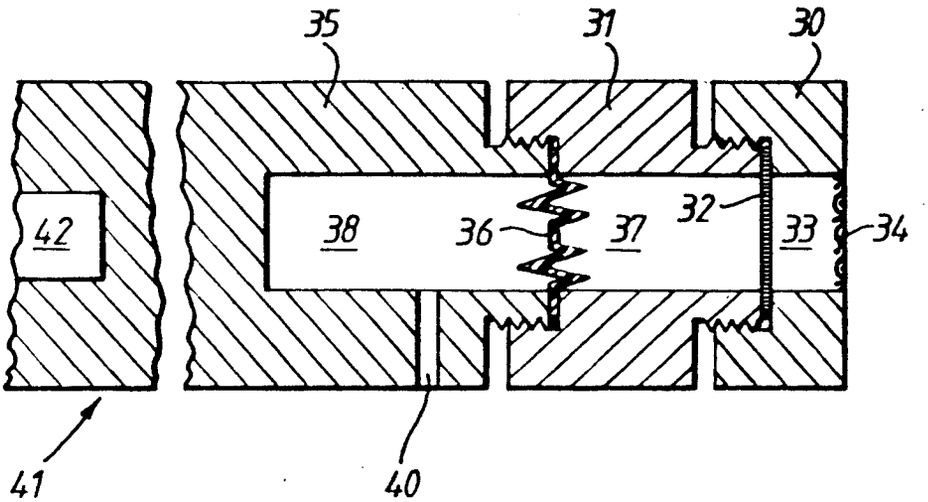

FIG. 2.

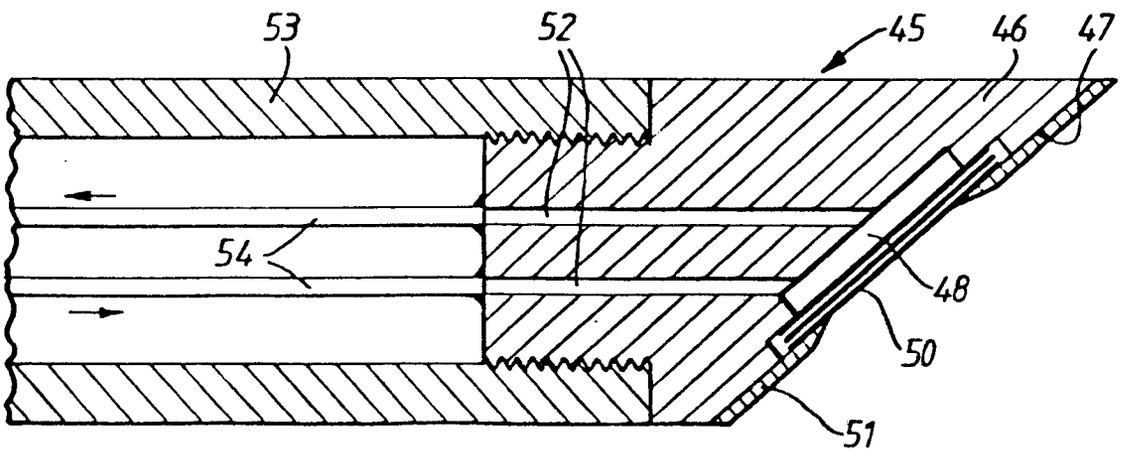

FIG. 3.

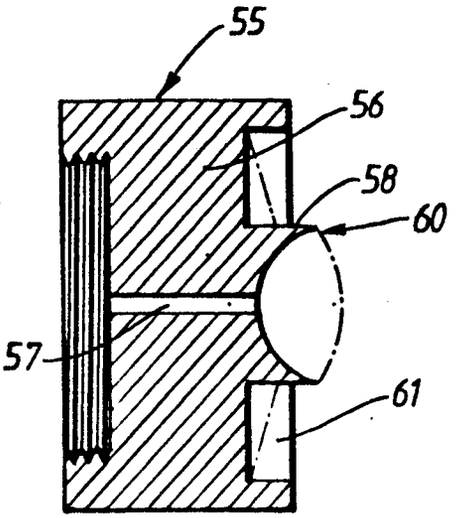

FIG. 4.

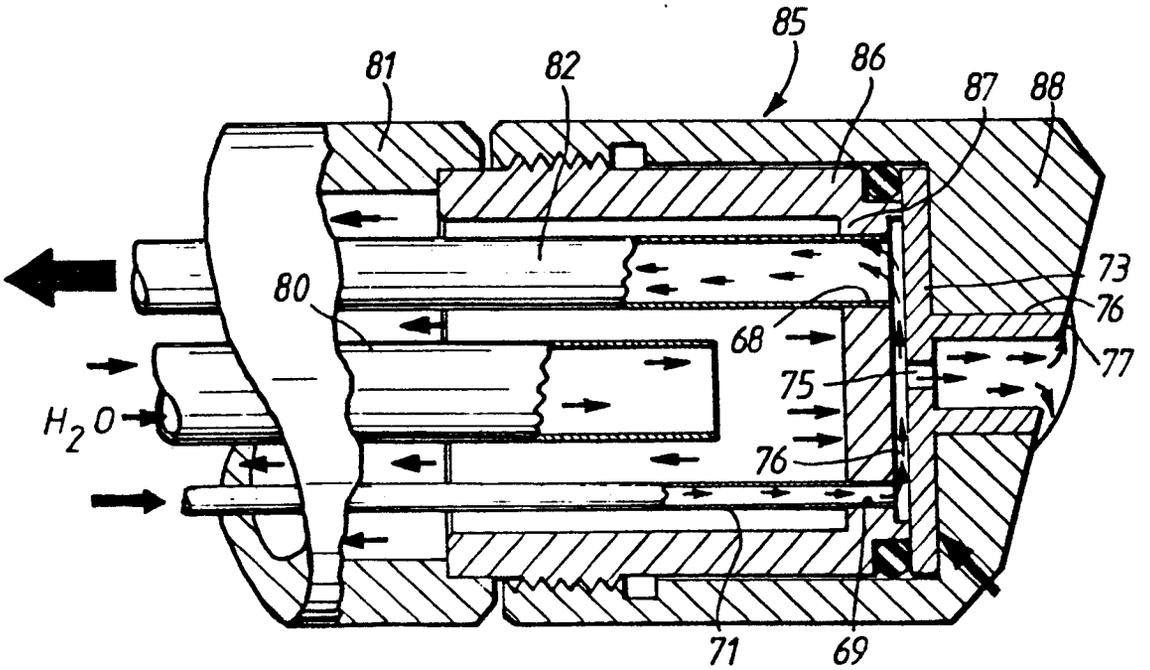

FIG. 5.