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(54) SHEATH LIQUID SUPPLYING APPARATUS, SHEATH LIQUID SUPPLYING METHOD, AND EVALUATING METHOD OF SHEATH LIQUID SUPPLYING CONDITION

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(52) **U.S. Cl.** **73/1.36**

(57) **ABSTRACT**

A sheath liquid supplying apparatus includes a syringe including a piston and a cylinder slidably accommodating the piston and a stepping motor for causing the piston to slide in the cylinder, wherein the cylinder has an injection/suction hole of a sheath liquid positioned at a distal end thereof and a gas introducing hole positioned at a side wall thereof for introducing gas into the cylinder.

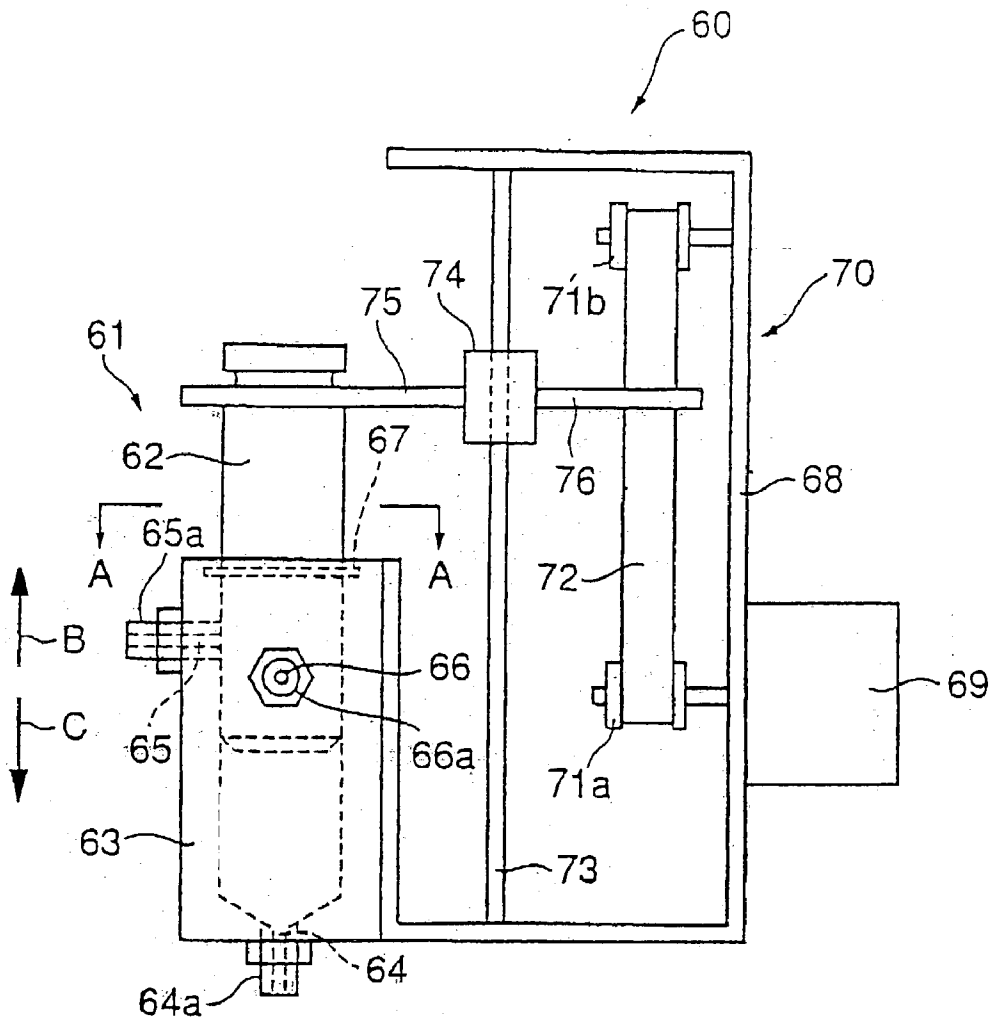


FIG. 1

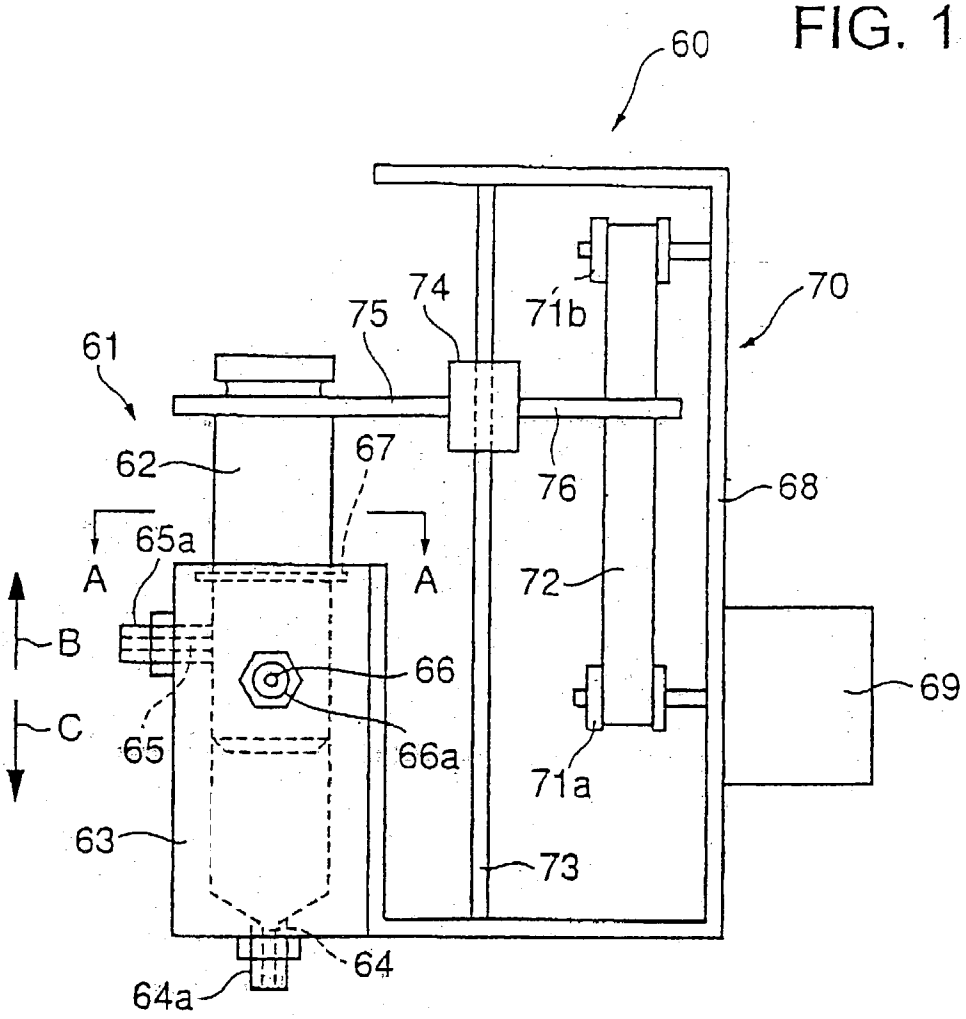

60
70
74
75
71b
61
62
67
76
68
65a
A
A
72
B
66
69
C
65
66a
71a
63
73
64
64a

FIG. 2

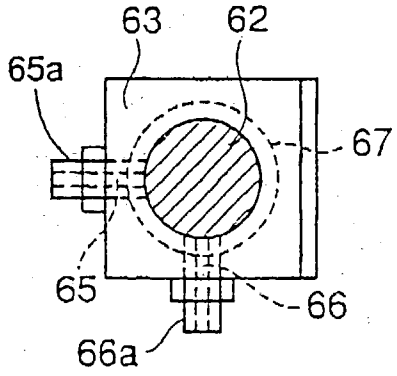

63
62
65a
67
65
66
66a

FIG. 3

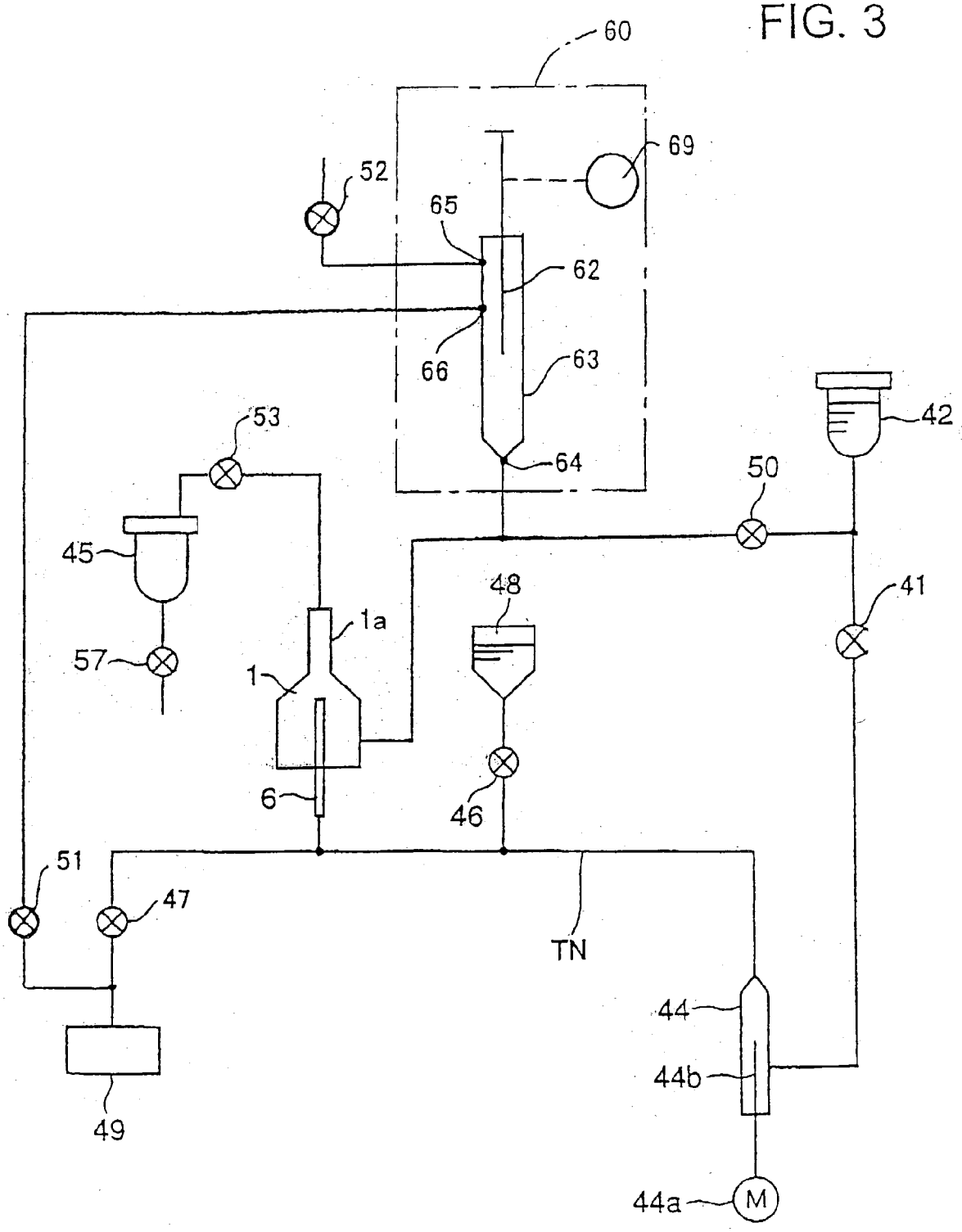

60
69
52
65
62
66
63
64
53
42
50
45
48
41
1a
57
1
6
46
51
47
TN
44
44b
49
44a
M

FIG. 4

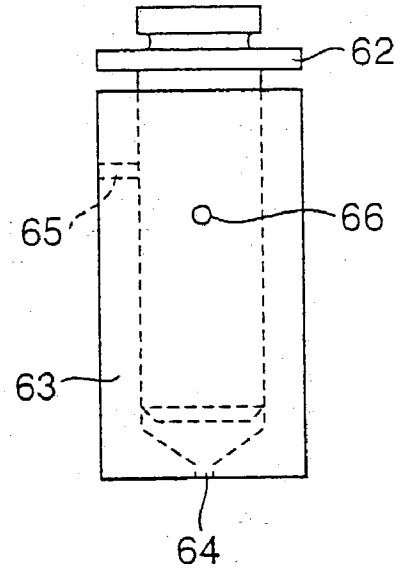


FIG. 5

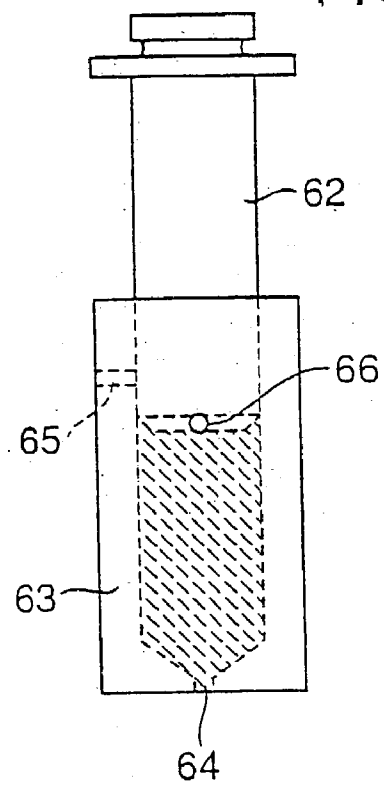


FIG. 6

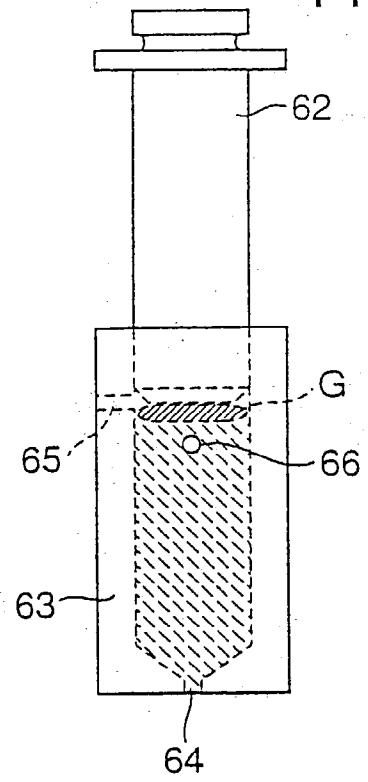


FIG. 7

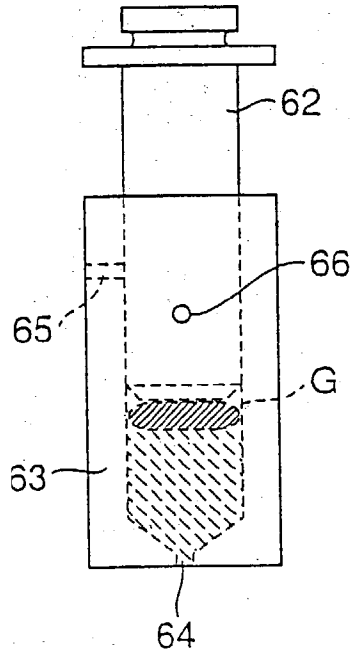


FIG. 8

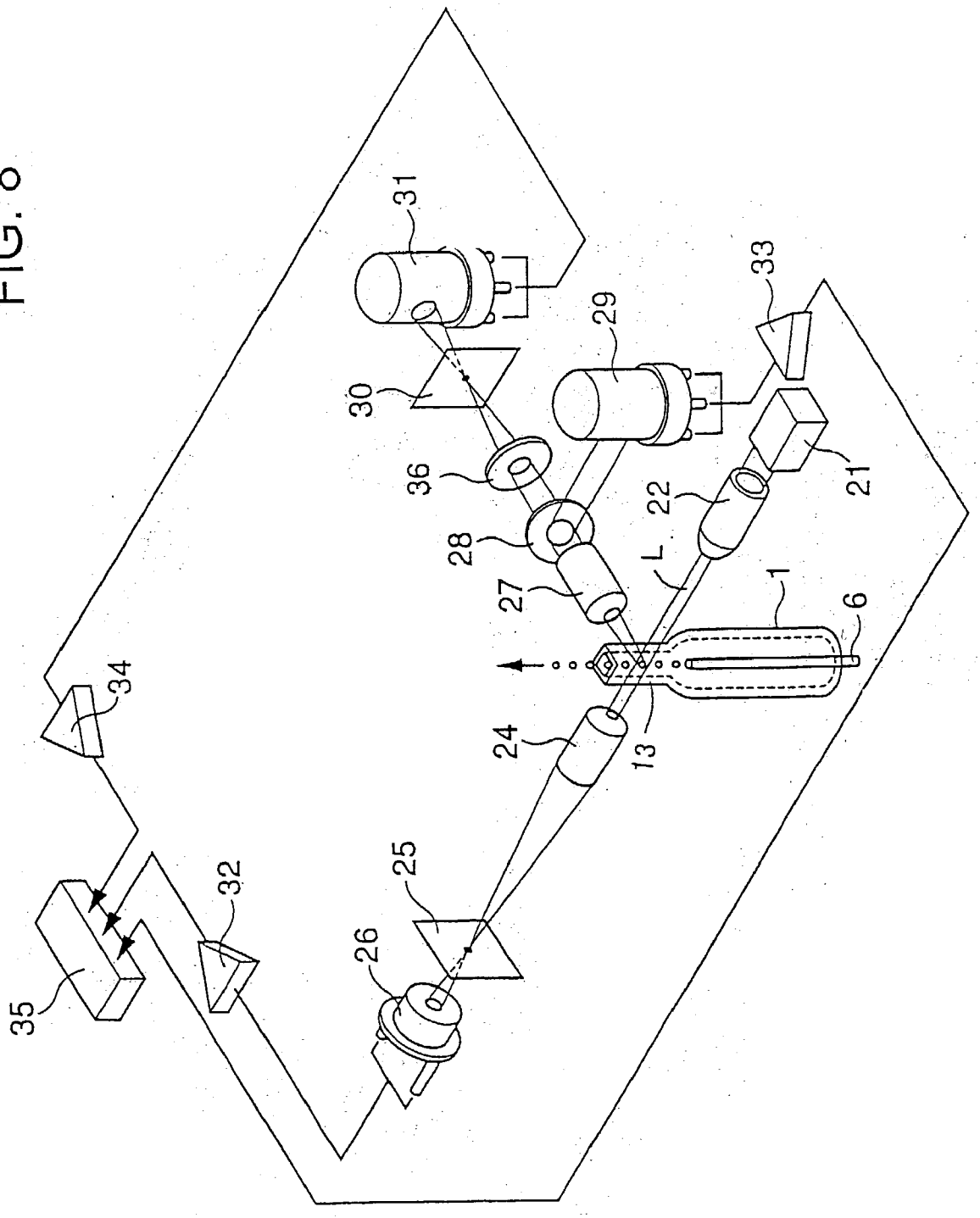


FIG. 9

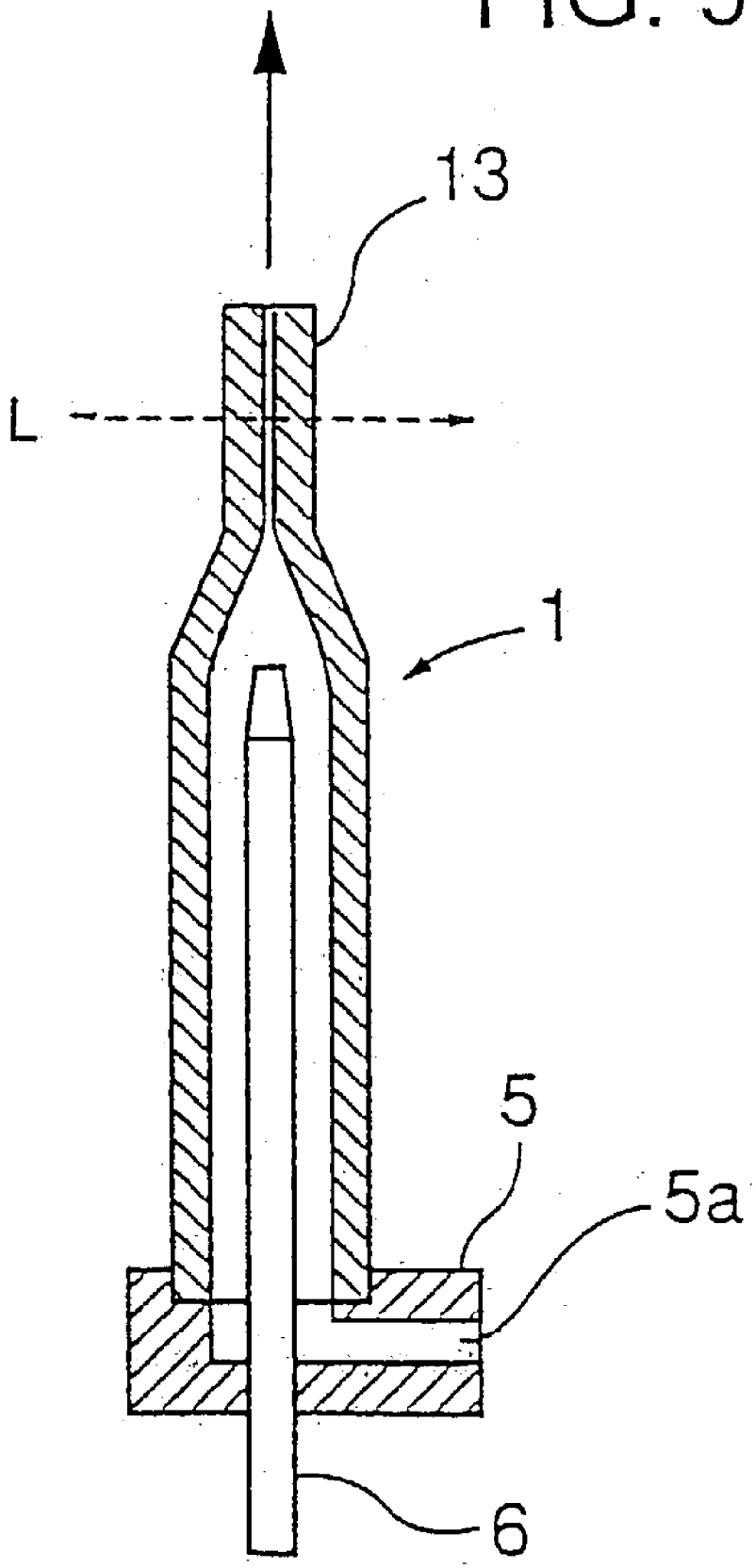


FIG. 10

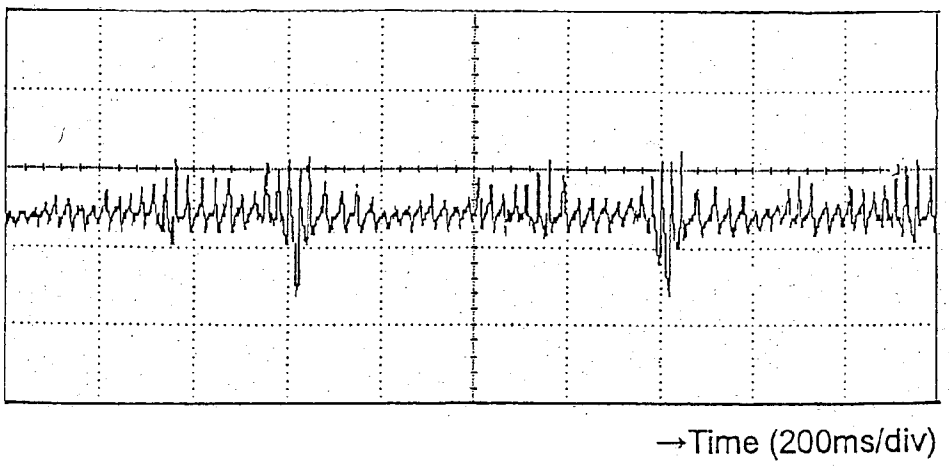


FIG. 11

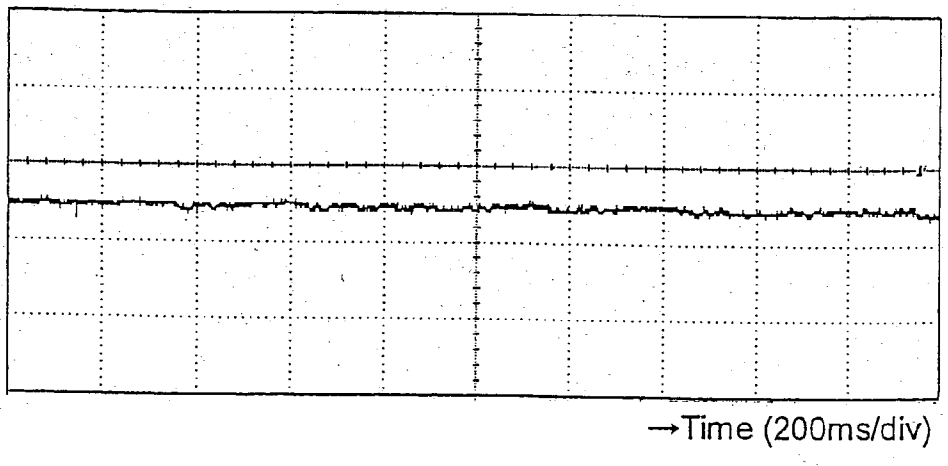
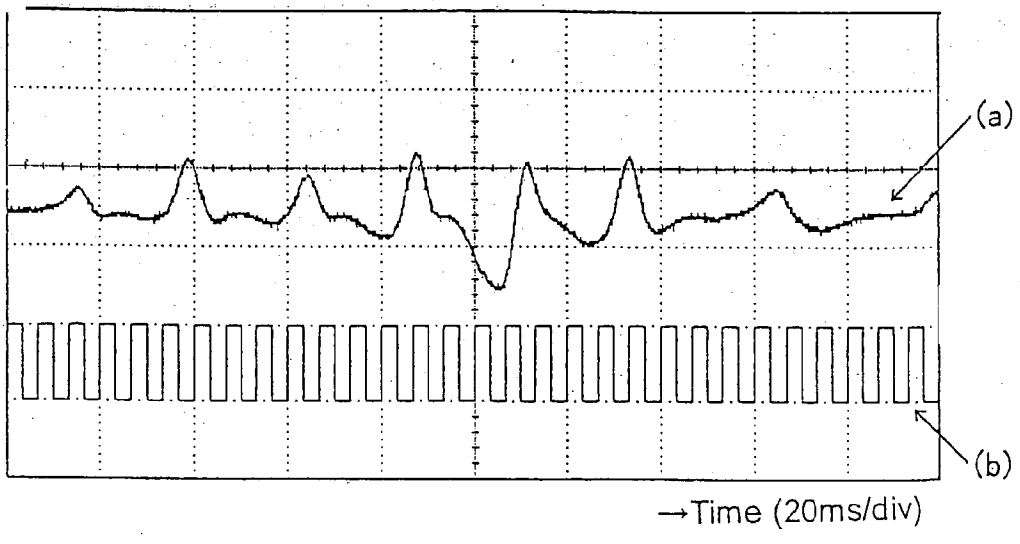


FIG. 12



SHEATH LIQUID SUPPLYING APPARATUS, SHEATH LIQUID SUPPLYING METHOD, AND EVALUATING METHOD OF SHEATH LIQUID SUPPLYING CONDITION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is related to Japanese Patent Application No. 2000-248689 filed in Aug. 18, 2000, whose priority is claimed under. 35 USC §119, the disclosure of which is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates to a sheath liquid supplying apparatus, a sheath liquid supplying method and an evaluating method of a sheath liquid supplying condition, and more particularly to an apparatus or method for supplying a sheath liquid to a sheath flow cell by using a syringe in a flow cytometer.

[0004] 2. Description of the Related Arts

[0005] A flow cytometer using a so-called sheath flow method has been well-known as an apparatus for analyzing particles such as a cell, blood cell, or the like in a sample. In this method, a sheath liquid is flown around a sample solution (particle-floating solution) ejected from a nozzle in a sheath flow cell to form a sheath flow, whereby the sample solution can be converged into a small flow in the sheath flow cell. The converged sample solution is optically measured for analyzing particles in the sample solution. The "sheath flow" means a flow for causing particles in the particle-floating solution (sample solution) pass therethrough in a line with precision by converging the sample solution into a small flow-having an outside diameter approximately same as that of the particle at the center portion of the sheath liquid that is flowing through an orifice in a laminar flow state. Analysis of various cells has been performed by using a sample solution obtained by adjusting a sample of blood or urine with a dyeing solution, hemolytic agent or reaction reagent.

[0006] The sample solution is supplied to the sheath flow cell by a syringe having high quantity accuracy. The sheath liquid is supplied to the sheath flow cell by using a method of applying a predetermined positive pressure (0.2-1.6 kgf/cm^2) to the sheath liquid chamber (referring to Japanese Unexamined Publication No. HEI 10(1998)-260129).

[0007] However, the supply of the sheath liquid to the sheath flow cell by using a method of applying the positive pressure to the sheath liquid chamber brings a change in viscosity of the sheath liquid in the case of changing the environmental temperature. This change in viscosity brings a change in flow velocity, thereby giving an adverse influence to the optical measurement.

[0008] Accordingly, this method requires a sheath liquid temperature adjusting function for maintaining a constant flow velocity of the sheath liquid, as well as requires an air compressor for applying the positive pressure to the sheath liquid chamber or a regulator for adjusting a pressure. This causes a problem that a pressure-adjusting and sheath liquid supplying apparatus is made complicated.

[0009] In case where the sheath liquid is supplied to the sheath flow cell by using a syringe that is driven by a stepping motor, the flow velocity is not affected by a change in the environmental temperature. However, the flow velocity of the sheath liquid changes depending upon a mechanical factor such as a pulse of a torque of the stepping motor, whereby a ripple occurs on the sheath liquid and sample solution in the sheath flow cell. Specifically, the sheath flow is brought into an unstable state. As a result, a ground noise of an optical detecting signal is fluctuated, which gives an adverse effect on the optical measurement.

SUMMARY OF THE INVENTION

[0010] The present invention is accomplished in view of the above circumstances, and aims to provide a sheath liquid supplying apparatus, a sheath liquid supplying method, a flow cytometer including said apparatus, and an evaluating method of a sheath liquid supplying condition wherein a sheath liquid is supplied by using a syringe that is driven by a driving motor. This construction provides that the flow velocity is not affected by an environmental temperature, and prevents a fluctuation in the flow velocity of the sheath liquid due to a mechanical factor such as a pulse of a torque of a stepping motor as well as prevents a fluctuation in a ground noise of an optical detecting signal brought with the fluctuation in the flow velocity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **FIG. 1** is a side view showing an embodiment of a sheath liquid supplying apparatus according to the present invention;

[0012] **FIG. 2** is a view in section taken along the line A-A in **FIG. 1**;

[0013] **FIG. 3** is a systematic view showing a fluid system of an analyzer of material components in urine that includes the sheath liquid supplying apparatus of the present invention;

[0014] **FIG. 4** is an explanatory view showing an operation of a syringe in the embodiment of the present invention;

[0015] **FIG. 5** is an explanatory view showing an operation of a syringe in the embodiment of the present invention;

[0016] **FIG. 6** is an explanatory view showing an operation of a syringe in the embodiment of the present invention;

[0017] **FIG. 7** is an explanatory view showing an operation of a syringe in the embodiment of the present invention;

[0018] **FIG. 8** is a systematic view showing an optical system of an analyzer of material components in urine that includes the sheath liquid supplying apparatus of the present invention;

[0019] **FIG. 9** is a sectional view showing a sheath flow cell of the analyzer of material components in urine to which the sheath liquid supplying apparatus of the present invention is adapted;

[0020] **FIG. 10** is a waveform view showing a performance of a comparative example;

[0021] **FIG. 11** is a waveform view showing a performance of the embodiment of the present invention; and

[0022] **FIG. 12** is an enlarged waveform view showing a performance of the comparative example.

DESCRIPTION OF THE PREFERRED EMBODIMENT

[0023] The present invention will be explained in detail with reference to the drawings. The present invention is not limited to the following explanation.

[0024] Construction of a Sheath Liquid Supplying Apparatus

[0025] **FIG. 1** is a side view showing an embodiment of a sheath liquid supplying apparatus according to the present invention and **FIG. 2** is a view in section taken along the line A-A, in **FIG. 1**.

[0026] As shown in these figures, a main body **60** of a supplying apparatus comprises a syringe **61** and a driving apparatus **70**. The syringe **61** has a piston **62** and a cylinder **63** that accommodates the piston **62** so as to be slidable in a direction shown by arrows B and C. The arrow B expresses up direction along the axial direction of the cylinder **63**, and the arrow C expresses down direction along the axial direction of the cylinder **63**. The piston **62** and cylinder **63** can be made of a material having a chemical resistance, such as glass, vinyl chloride, stainless steel or the like. It is necessary for the syringe **61** to have a capacity, which can at least supply a sheath liquid required for one measurement in a sheath flow cell. For example, the capacity may be approximately 3 to 5 mL.

[0027] The cylinder **63** is provided with an injection/suction hole **64** of the sheath liquid at its bottom edge that injects the sheath liquid to the cylinder **63** and extracts the sheath liquid from the cylinder **63** corresponding to the forward and rearward motions of the piston **62**. Provided also at the cylinder **63** are a gas introducing hole **65** and a negative pressure introducing hole **66** at its side wall. The gas introducing hole **65** introduces a gas (for example, an air) between a liquid surface of the sheath liquid accommodated in the cylinder **63** and the piston **62** to form a gas layer. A buffer action of the gas layer absorbs a rotational irregularity (periodical fluctuation of a torque), to thereby stabilize the ejecting velocity of the sheath liquid. Accordingly, the gas introducing hole **65** is mounted in the vicinity of a piston inserting opening of the cylinder **63**.

[0028] A circular seal member **67** is disposed at the upper inside surface of the cylinder **63** for sealing up the inside surface of the cylinder **63** and the outside surface of the piston **62**.

[0029] The negative pressure introducing hole **66** is provided closer to the injection/suction hole **64** compared to the gas introducing hole **65** with respect to the axial direction of the cylinder **63**. Nipples **64***a*, **65***a* and **66***a* for connecting an external tube are respectively disposed at the injection/suction hole **64**, gas introducing hole **65** and negative pressure introducing hole **66** of the syringe **61**.

[0030] A driving apparatus **70** has a frame **68**, a stepping motor **69** mounted to the frame **68**, a driving pulley **71***a* mounted to an output shaft of the stepping motor **69**, a follower pulley **71***b* rotatably supported by the frame **68** and an endless belt **72** bridged between the pulleys **71***a* and **71***b*.

[0031] The frame **68** has a slide shaft **73** mounted along the axial direction of the cylinder **63** that supports a sliding member **74** so as to be capable of sliding in the direction shown by arrows B and C. The sliding member **74** has arms **75** and **76** which horizontally project to respectively connect to the upper edge of the piston **62** and the endless belt **72**.

[0032] When the stepping motor **69** rotates, its rotational motion is converted into the linear motion by the pulley **71***a*, **71***b* and the endless belt **72**. The linear motion is transmitted to the piston **62** via the arm **76**, sliding member **74** and arm **75**, whereby the piston **62** can be driven in the directions of B and C.

[0033] A motor now on sale can be used as the stepping motor **69**. For example, the stepping motor of PK43 AG470-100(12)6TA-3 manufactured by Sanryu Co., Ltd. can be used as the stepping motor **69** causing the piston **62** to slide in the cylinder **63**.

[0034] Analyzer of Material Components in Urine to which Sheath Liquid Supplying Apparatus is Adapted

[0035] **FIG. 3** is a systematic view showing a flow system where the sheath liquid supplying apparatus in **FIG. 1** is adapted to an analyzer of material components in urine. The analyzer is a so-called flow cytometer. Each component of the flow system is connected by flow path of a tube network TN.

[0036] A sheath flow cell **1** in this analyzer of material components in urine has a construction shown in **FIG. 9**. A sheath liquid is supplied from the sheath liquid supplying apparatus **60** to an injection hole **5***a* of a sheath liquid injecting section **5**. A sample liquid is supplied to a nozzle **6** from a syringe **44** for supplying a sample liquid.

[0037] In an initial condition, the sheath liquid supplying apparatus **60** is in a condition where the leading edge of the piston **62** nearly reaches the injection/suction hole **64** at the bottom of the cylinder **63** as shown in **FIG. 4**. When a valve **50** is opened to lift the piston **62** in the direction of B (see **FIG. 1**), the sheath liquid in an open-air sheath liquid chamber **42** is sucked in the cylinder **63** via a valve **50**. When the leading edge of the piston **62** reaches the vicinity of the negative pressure introducing hole **66** as shown in **FIG. 5**, the piston **62** temporarily stops rising, and then, a valve **51** is opened.

[0038] By this operation, a negative pressure of a suction apparatus **49** is applied to the inside of the cylinder **63** via the negative pressure introducing hole **66**, whereby the sheath liquid is sucked into the suction apparatus **49** via the chamber **42**, valve **50**, injection/suction hole **64**, negative pressure introducing hole **66** and valve **51**, so that bubbles are eliminated from the sheath liquid sucked into the cylinder **63**.

[0039] Subsequently, the valve **51** is closed and the valve **52** is opened, with the result that the piston **62** moves upward until its leading edge passes a little through the gas introducing hole **65**. By this operation, air is introduced from the gas introducing hole **65** to thereby form an air layer G (hereinafter referred to as air damper) between the sheath liquid surface and the leading edge of the piston **62**.

[0040] After the air damper having a predetermined volume is formed, the piston **62** terminates, and the valves **50** and **52** are closed. The sheath liquid supplying apparatus **60**

finishes here the preparation for supplying the sheath liquid. A washing process and measuring process are executed as follows.

[0041] Washing Process

[0042] Firstly, valves **41**, **47** and **50** are opened for sucking the sheath liquid with the negative pressure of the suction apparatus **49** from the open-air sheath chamber **42** accommodating the sheath liquid. The sheath liquid is discharged to the suction apparatus **49** via the valve **50**, sheath flow cell **1**, nozzle **6** and valve **47**, and at the same time, discharged to the suction apparatus **49** via a metering syringe **44** for supplying a sample liquid and the valve **47**. Thereafter, the valves **41**, **47** and **50** are closed after a predetermined period. By this operation, the metering syringe **44**, nozzle **6**, sheath flow cell **1** and its flow path are washed with the sheath liquid.

[0043] Measuring Process

[0044] Subsequently, valves **46** and **47** are opened for sucking a sample liquid with the negative pressure of the suction apparatus **49** from a reaction chamber **48** in which a sample liquid containing material components in urine is reacted with a reactant and the resultant is accommodated. When the sheath liquid in the flow path between the valve **46** and the nozzle **6** is replaced with the sample liquid, the valves **46** and **47** are closed.

[0045] Next, the valve **53** is opened and the stepping motor **69** of the sheath liquid supplying apparatus **60** is driven for moving the piston **62** toward the injection/suction hole **64** as shown in **FIG. 7**. By this operation, the sheath liquid in the cylinder **63** is supplied to the sheath flow cell **1** via the injection/suction hole **64**, whereby it is injected to the injection hole **5***a* of the sheath liquid injecting section **5** in the sheath flow cell **1**.

[0046] Subsequently, a piston **44***b* of the metering syringe **44** is driven by a motor **44***a*, whereby the sample liquid present between the valve **46** and the nozzle **6** is ejected from the nozzle **6** as shown in **FIG. 9**. The ejected sample liquid is converged into a small flow with the sheath liquid for passing through an orifice **13**, and then, discharged to an open-air discharge liquid chamber **45** with the sheath liquid.

[0047] A laser beam L is irradiated to the orifice portion **13** as described later for optically measuring material components in urine among the sample liquid. Thereafter, the piston **44***b* of the metering syringe **44** is driven during a predetermined period to supply the predetermined amount of sample liquid to the sheath flow cell **1**. Then, the valve **53** is closed to finish the measuring process. The valve **57** is opened, as necessity requires, for discharging the sheath liquid and sample liquid accommodated in the discharge liquid chamber **45**.

[0048] In the above measuring process, the sheath liquid is pushed by the piston **62** driven by the stepping motor **69** to be supplied from the cylinder **63** to the sheath flow cell **1**. The stepping motor originally has a rotational irregularity (periodical fluctuation of torque). This rotational irregularity is absorbed by a buffer operation of the air damper G shown in **FIG. 7**. As a result, the sheath liquid is smoothly supplied to the sheath flow cell **1** with a constant flow velocity without generating a fluctuation in flow velocity as shown in the result of a performance test described later.

[0049] When the measuring process is finished in this way, the process for injecting the sheath liquid to the cylinder **63** of the sheath liquid supplying apparatus **60** and the washing process are performed to make preparations for the next process.

[0050] **FIG. 8** is a perspective view showing an optical system of the analyzer of material components in urine. In the same figure, a laser beam L emerged from a laser diode **21** irradiates the orifice portion **13** of the sheath flow cell **1** via a collimator lens **22**. The forward scattered light emerging from the material components in urine which pass through the orifice portion **13** is incident to a photodiode **26** via a focusing lens **24** and a pinhole plate **25**.

[0051] On the other hand, the sideward scattered light emerging from the material components in urine which pass through the orifice portion **13** is incident to a photomultiplier tube (hereinafter referred to as photomul) via a focusing lens **27** and a dichroic mirror **28**, while the sideward fluorescence emerging from the material components in urine which pass through the orifice portion **13** is incident to a photomul **31** via the focusing lens **27**, dichoric mirror **28**, a filter **36** and a pinhole plate **30**.

[0052] The forward scattered light signal outputted from the photodiode **26**, the sideward scattered light signal outputted from the photomul **29** and the sideward fluorescence signal outputted from the photomul **31** are respectively amplified by each amplifier **32**, **33** and **34**, and then, inputted to an analyzing section **35**. The analyzing section **35** is comprised of a microcomputer that processes and analyzes the output signals from the photodiode **26**, photomul **29** and **31** based upon a predetermined program and outputs the resultant to a display device or a printer.

[0053] Performance Test of Sheath Liquid Supplying Apparatus

[0054] A fluctuation in flow velocity (irregularity in flow velocity) of the sheath liquid in the sheath liquid supplying apparatus **60** can be examined by the following manner by using the analyzer of material components in urine shown in **FIGS. 3 and 8**.

[0055] Firstly, a liquid having a refractive index N_T is prepared as a sheath liquid, while a liquid having a refractive index N_3 (not equal to N_T) is prepared as a sample liquid.

[0056] Subsequently, prepared each liquid is supplied to the sheath flow cell **1** by using the flow system shown in **FIG. 3**. The laser beam L is irradiated to the orifice portion **13** of the sheath flow cell **1** with the optical system of **FIG. 8**. The photodiode **26** detects its scattered light intensity and the detected light is amplified by the amplifier **32**.

[0057] The amplified signal waveform is observed by an oscilloscope.

[0058] When the ripple occurs in the sample liquid flow at the orifice portion **13** due to the fluctuation in flow velocity of the sheath liquid, the scattered light intensity from the laser beam L changes because of the difference in refractive index between the sheath liquid and the sample liquid flow, whereby the output signal from the amplifier **32** fluctuates (it is considered that the fluctuation in the output signal depends upon "each refractive index of sample liquid and sheath liquid" and "a width of ripple in sample liquid flow").

[0059] In view of this, liquids each having the following refractive indices N_T and N_S were prepared as the sheath liquid and sample liquid.

[0060] N_T=1.341

[0061] N_S=1.334

[0062] Note that liquids each having a different refractive index can be adjusted, for example, with solutions of salt each having a different concentration.

[0063] The flow amount of the sample liquid was set to 1.7 μL/seconds, while the flow velocity of the sample liquid was set to 7.5 m/seconds that can obtain a laminar flow. The output waveform from the amplifier **32** was recorded with the oscilloscope with respect to the presence of the air damper G **(FIGS. 6 and 7)**. The results are shown in **FIGS. 10 and 11**. **FIG. 10** represents the case where the air damper G is not formed, while **FIG. 11** represents the case where the air, damper G is formed.

[0064] In **FIG. 12, a** waveform (a) is obtained by recording the waveform in **FIG. 10** with a tenfold time axis, while a waveform (b) represents a waveform of a driving pulse of the stepping motor **69 (FIG. 1)** corresponding to the waveform (a).

[0065] It is understood from **FIG. 10** that the waveform has a large fluctuation (ripple) to cause a great ripple in the sample liquid flow at the orifice portion **13** in case where the air damper G is not formed. On the other hand, it is understood from **FIG. 11** that the waveform has a small fluctuation to thereby prevent the ripple from occurring in the sample liquid flow in case where the air damper G is formed. Consequently, the formation of the air damper G brings a stable flow of the sample liquid.

[0066] **FIG. 12** represents that a main cause of the fluctuation in the waveform (a) is caused by the rotational irregularity (periodical fluctuation in torque) of the stepping motor **69** since the correlation is periodically established between the waveform (a) and the waveform (b). **FIG. 10** represents that the rotational irregularity is effectively absorbed by the air damper G.

[0067] It is understood that the minimum volume necessary for the air damper G may be set to the one that brings a minimum amplitude of the waveform of **FIG. 10**.

[0068] According to a sheath liquid supplying apparatus and its method of the present invention, the supplying apparatus itself can simply be realized with a combination of a syringe and a stepping motor. Further, gas is intervened between a leading edge of a piston in the syringe and a sheath liquid in the cylinder for absorbing a fluctuation of the piston due to the stepping motor, whereby the sheath liquid can be supplied to a sheath flow cell with a constant stable speed.

[0069] Moreover, according to an evaluating method of a sheath liquid supplying condition, liquids each having a different refractive index are supplied to a sheath flow cell as a sheath liquid and a sample liquid, and a degree of the fluctuation in the scattered light intensity upon irradiating light to the sheath flow cell teaches information such as a periodical change, fluctuation period, fluctuation width or the like can be obtained with respect to the supplying condition of the sheath liquid. Specifically, it is possible to simplify the evaluating method.

What is claimed is:

1. A sheath liquid supplying apparatus comprising:

a syringe including a piston and a cylinder slidably accommodating the piston and

a stepping motor for causing the piston to slide in the cylinder,

wherein the cylinder has an injection/suction hole of a sheath liquid positioned at a distal end thereof and a gas introducing hole positioned at a side wall thereof for introducing gas into the cylinder.

2. A sheath liquid supplying apparatus claimed in claim 1, wherein the cylinder has a negative pressure introducing hole positioned at a side wall thereof for introducing a negative pressure into the cylinder.

3. A sheath liquid supplying apparatus claimed in claim 2, wherein the negative pressure introducing hole is positioned closer to the injection/suction hole compared to the gas introducing hole with respect to an axial direction of the cylinder.

4. The sheath liquid supplying apparatus claimed in claim 1, wherein the piston or cylinder is glass, vinyl chloride or stainless steel.

5. The sheath liquid supplying apparatus claimed in claim 1, wherein the syringe has a capacity of about 3-5 mL.

* * * * *