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Bean et al.

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[54] **PARTICLE ANALYZER**

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[73] Assignee: **General Electric Company,** Schenectady, N.Y.

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[21] Appl. No.: **228,719**

Related U.S. Application Data

[63] Continuation of Ser. No. 13,115, Feb. 20, 1970, abandoned.

[52] **U.S. Cl.** ..... **324/71 CP,** 204/299, 73/432 PS

[51] **Int. Cl.** **G01n 27/00**

[58] **Field of Search** ..... 324/30 R, 30 B, 71 PC; 356/105; 204/180 R, 299; 73/432 PS

[56] **References Cited**

UNITED STATES PATENTS

3,208,919	9/1965	Sennet	324/30 R
3,395,344	7/1968	Bader	324/71 PC
3,441,848	4/1969	Valley	324/71 PC
3,454,487	7/1969	Riddick	204/299

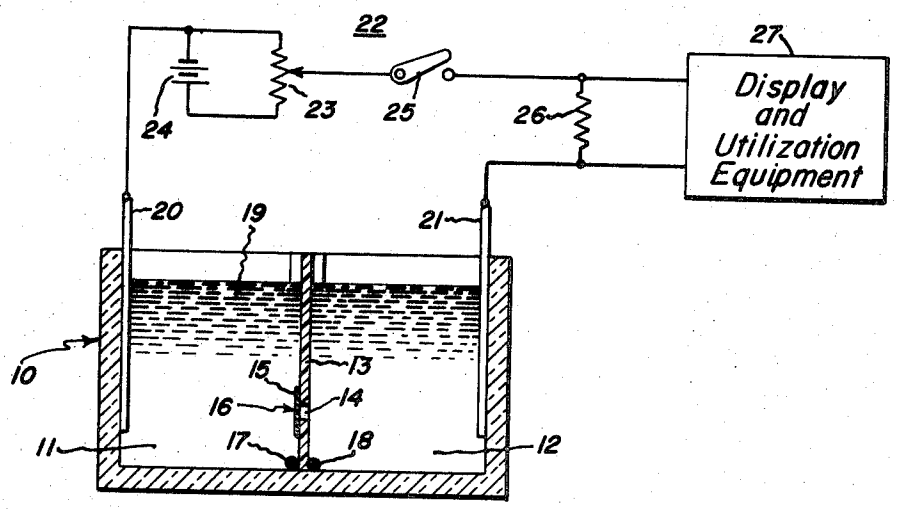
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[57] **ABSTRACT**

An apparatus and method for electrophoretically driving solution-suspended small particles through a pore in a membrane and means for counting, sizing and characterizing the particles is disclosed. The apparatus comprises a container having two chambers fitted with electrodes, filled with an electrolyte solution and separated by a partition having a membrane with a pore through which small particles are driven by an electric field. The size, number and mobility of these particles is determined by appropriate apparatus.

5 Claims, 4 Drawing Figures



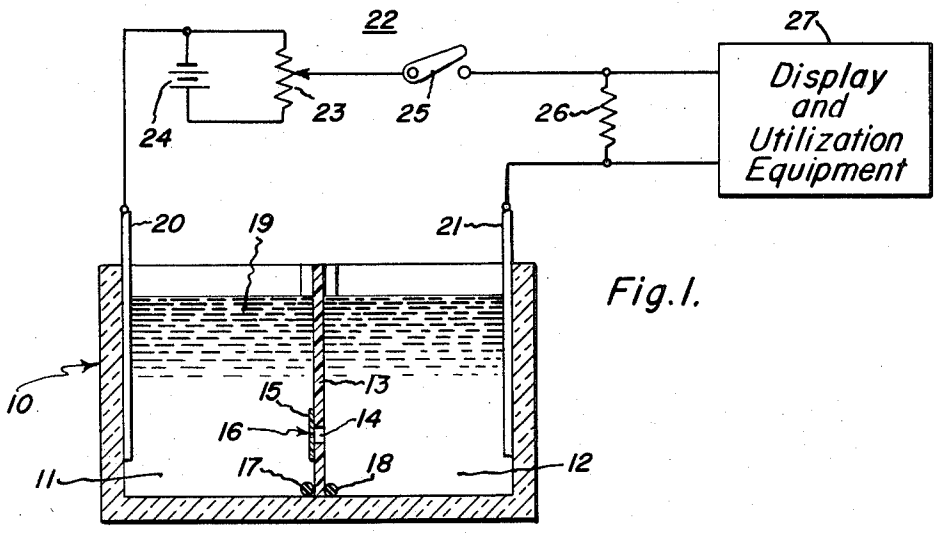


Fig. 1.

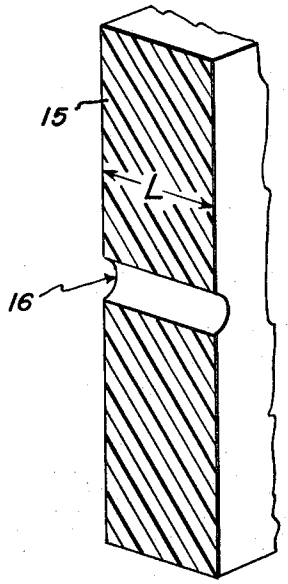


Fig. 2.

Fig. 4.

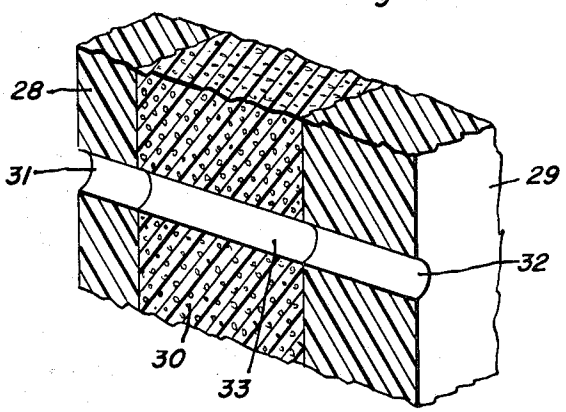
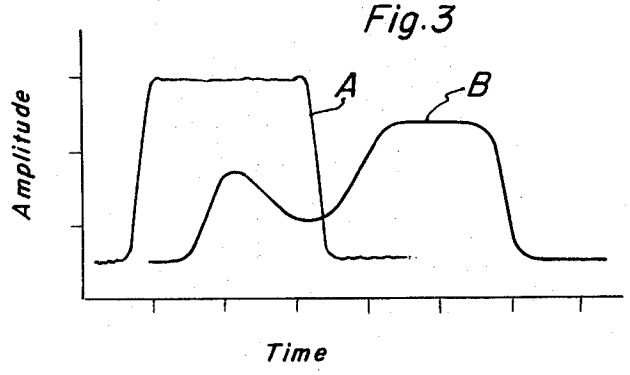


Fig. 3



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PARTICLE ANALYZER

This is a continuation of Ser. No. 13,115, filed Feb. 20, 1970, now abandoned.

The present invention relates to a method and apparatus for analyzing small particles and more particularly to a novel method and apparatus for counting, sizing and characterizing colloidal-size particles suspended in a liquid.

The need to count and size individual microscopic particles exists in a wide variety of fields. For example, in the medical field, it is desirable and often necessary to count and size blood cells. One such device which performs this function is described in U.S. Pat. No. 2,656,508 to Coulter. Basically, the Coulter counter is an instrument which sizes and counts small particles suspended in an electrolyte by passing these particles from one chamber to another through a small current-carrying aperture by means of a pressure difference therebetween. The passage of particles through the aperture increases the resistance thereof by displacing some of the liquid. This change in resistance may be detected as an increase in voltage across the aperture or as a decrease of current through the aperture. Since the change in resistance is also proportional to the volume of the particle passing through the aperture, with proper instrumentation, the size-number distribution of particles in a given sample can be obtained.

Present day particle analyzers are generally limited to the measurement of particles greater than 0.5 micrometers or more in diameter. This limitation results primarily from the difficulties encountered in making very small apertures and from the coagulation of particles in the aperture with resultant plugging or clogging thereof. Still a further characteristic of present day analyzers is the need for a pressure difference between the chambers of the analyzer so that the particles are driven through the aperture by the pressure difference.

By virtue of the present invention, which is predicated upon a novel discovery, subsequently to be described in detail, particles of up to about 10 times smaller in diameter can be counted and sized by driving the particles through extremely small apertures or pores by using an electric field rather than a pressure difference. Since particles suspended in a liquid, in general, have a net electric charge which is related, among other things, to the surface properties of the particles, it is now possible to use this charge to characterize or distinguish one particle from another. More specifically, the charge of a particle is a factor in determining how rapidly the particle will pass through a pore under the influence of an electric field, hence it is now possible to determine the mobility of a particle by measuring the time of passage through the pore. This new parameter is of particular significance for distinguishing between two different type particles of the same size or for categorizing a sample of particles according to number, size and mobility, i.e., the velocity of movement of a particle divided by the applied electric field strength, generally measured in cm^2/volt seconds.

It is therefore an object of this invention to provide apparatus for analyzing particles according to number, size and mobility.

It is another object of the instant invention to provide apparatus for categorizing particles of sub-micron size.

It is a further object of the instant invention to provide apparatus for electrically driving particles through very small apertures.

It is still a further object of this invention to provide apparatus for categorizing particles which is less susceptible to clogging or plugging problems of the prior art.

These and other objects of this invention are achieved in one embodiment of the invention by providing two chambers separated by a partition having an opening therein covered by a thin membrane with a pore passing therethrough for providing communication between the two chambers. Each chamber is fitted with an electrode and filled with an electrolyte solution. An electric potential is applied between the electrodes in series with a load impedance so as to provide a suitable voltage gradient across the pore for driving particles therethrough. The particles to be sized, counted and categorized according to mobility are then added to one of the chambers and electrically driven through the pore. The size, number and mobility of the particles are determined by appropriate measuring apparatus connected to the load impedance.

The novel features believed characteristic of the invention are set forth in the appended claims. The invention itself, together with further objects and advantages thereof may best be understood by reference to the following description, taken in connection with the accompanying drawing, in which:

FIG. 1 is an illustration of suitable apparatus for practising this invention;

FIG. 2 is a greatly enlarged view of a membrane with a pore passing therethrough;

FIG. 3 is an illustration of typical amplitude vs. time characteristics of signals resulting from the passage of particles through different length pores under the influence of an electric field; and

FIG. 4 is an enlarged view of an alternate embodiment of a membrane having an extended pore and also useful in practising the instant invention.

By way of example, FIG. 1 illustrates an embodiment of the invention comprising a container or cell **10** divided into chambers **11** and **12** by a partition **13.** The container **10** and partition **13** are preferably formed of a substantially non-porous insulating material such as ceramic, plastic, glass or plexiglass. The partition **13** has an aperture **14** therein covered by a membrane **15** with a pore **16** of a suitable size produced therein in a manner to be described hereinafter. The membrane **15** is attached to the partition **13** by suitable adhesion means such as epoxy cement, glue or other suitable adhesives. The partition **13** is mounted to the walls and bottom of the container **10** by O-rings **17** and **18,** for example. Other means for mounting the partition **13** can also be employed; however, it is preferable that the partition **13** be removable so that membranes having different size pores may be employed, if desired.

The compartments **11** and **12** of container **10** are filled with an electrolyte solution **19** such as potassium chloride, sodium chloride, dilute hydrochloric acid, sodium hydroxide or any of the other electrolyte solutions well known in the art, including buffering additives, if desired. The solution levels in the two compartments are adjusted to be substantially the same so that substantially no pressure difference exists therebetween. To provide conduction through the electrolyte solution **19,** electrodes **20** and **21** are positioned within

the chambers **11** and **12,** respectively. An external electrical circuit **22** comprising a variable voltage source such as a battery **24** in parallel with a rheostat **23,** a switch **25** and a load impedance **26** such as a resistor, are serially connected between the electrodes **20** and **21.** Upon closing the switch **25,** an electrical current is permitted to flow through the electrical circuit **22** and the electrolyte solution **19** by way of the pore **16.** Since the pore **16** is preferably of sub-micron or micron diameter, the flow of current therethrough is impeded by the resistance of the pore, generally designated by R_p. If the pore **16** has a diameter D and a length L and the electrolyte solution **19** has a resistivity ρ, then the resistance R_p for L much greater than D approaches:

$$R_p \approx 4\rho L/\pi D^2 \quad (1)$$

As will be more fully appreciated from the description hereinafter, pore resistances of several megohms to tens of megohms are not uncommon. Accordingly, to monitor the current flow through the pore **16,** it is desirable that load impedance **26** have a substantially high value, such as 1 megohm or greater. The voltage drop across the load impedance **26** can conveniently be monitored by display and utilization equipment **27** which may typically comprise an oscilloscope, voltmeter, peak amplitude detector, counter or other suitable monitoring and indicating devices well known in the art.

As previously noted, the membrane **15** is provided with at least one pore **16,** preferably of sub-micron or micron range so that the full advantages and benefits of the present invention are realized. To produce pores in this range which are of substantially uniform diameter and aligned preferably perpendicular to the major surface of the membrane, it is preferred that the method described and claimed in the Price and Walker patent, U.S. Pat. No. 3,303,085, be employed; briefly, this method comprises the steps of irradiating a material to produce tracks of structural damage caused by the irradiating particles and then etching the material in a solution which attacks the damaged tracks at a higher rate than the undamaged material.

In accord with a preferred embodiment of the instant invention, the membrane **15** is preferably an electrically insulating material having a thickness of less than 20 microns. In general, glass, mica or any synthetic resin, e.g., polyester resins such as polycarbonates and polyethylene terephthalate, as well as cellulostic materials such as cellulose nitrate, cellulose acetate, and mixtures thereof, are suitable for utilization as the membrane in the practise of the instant invention, although polycarbonate resin sheets are preferred because of the susceptibility of polycarbonate resins to fission track damage by heavy fission fragments and the accelerated etch rate of the fission tracks in polycarbonate resins relative to undamaged regions of the resin sheet.

Although the thickness of the membrane has been indicated to be preferably less than 20 microns, other factors are to be taken into consideration in determining the thickness thereof. For example, as illustrated in FIGS. **1** and **2,** the thickness of the membrane generally corresponds to the length L of the pore. As will become more apparent from the following detailed description of operation of the instant invention, it is preferable that the length of the pore to the diameter of the pore be approximately 5:1. Such a relationship between the length and diameter of the pore produces a signal at the load impedance **26** which has attained a steady state signal condition, i.e., transient conditions have settled. However, it can be readily appreciated by those skilled in the art that length to diameter ratios of as low as 2:1 and higher than 5:1 can be employed if desired or necessary. Accordingly, the appended claims are intended to encompass these various ratios.

In accord with the embodiment of the invention illustrated in FIG. **1,** colloidal size particles (less than one micron) are sized, counted and categorized according to transit time or mobility, if desired, by adding the particles to the electrolyte solution **19** in the chamber **11.** As the particles disperse in the electrolyte solution, eventually some of the particles will arrive at the vicinity of the pore **16.** With the switch **25** in a closed position, electrical current flowing through the electrolyte solution and the pore **16** establishes a potential gradient across the length of the pore. For example, a pore having a diameter of 0.45 microns (4500 A.) and an effective length of 3.3 microns may exhibit a voltage drop between the electrodes of 0.343 volts in a solution having a resistivity of 75 Ω cm. The voltage strength or field gradient, using equation (1) and Ohm's Law, is then equal to 0.1120 volts per micron or 1120 volts per centimeter. With such a field gradient across the length of the pore, particles in the vicinity of the pore entrance are quickly swept through the pore and effect an increase in resistance of the pore during the time of passage therethrough. For particles having diameters less than 0.4 of the diameter of the pore, the change in resistance of the pore is proportional to the cube of the diameter of the particle divided by the fourth power the diameter of the pore. More specifically, the change in resistance of the pore during the passage of a particle therethrough, is given by the following equation:

$$\Delta R = 4\rho d^3/\pi D^4 \quad (2)$$

where ΔR is the change in resistance, ρ is the resistivity of the electrolyte solution, d is the diameter of the particle and D is the diameter of the pore and where d *24* 0.4 D.

The particles passing through the pore **16** produce a decrease in voltage across the load impedance **26** during the interval of passage through the pore. The signal produced by the passage of a particle through the pore **16** is illustrated graphically by curve A of FIG. **3** wherein the sloping portions of the curve illustrate the entrance into and the exit from the pore and the substantially flat portion of the pulse between the sloping portions indicates the passage through the central portion of the pore. The amplitude of the pulse depends on the diameter of the particle and the pore in the manner described above and the pulse width is equal to the time of passage through the pore.

The voltage signal developed across the load impedance **26** can be used for indicating the number and size of the particles passing through the pore. For example, with the passage of each particle through the pore **16,** a voltage pulse substantially similar to that illustrated by curve A in FIG. **3** is produced across the load impedance **26.** This pulse can be counted by any of the numerous pulse counting devices well known in

the art. Additionally, since the amplitude of the pulse depends on the size of the particle and the diameter of the pore in accord with equation (2), knowing the diameter of the pore, the size of the particles can be readily determined. Equipment for performing this function is also well known in the art.

The embodiment of the invention illustrated in FIG. 1 therefore enables the counting and sizing of particles by electrophoretically driving the particles through a pore without the use of pressure variations between the two chambers. As described previously, this feature is particularly significant since in addition to enabling the counting and sizing of extremely small particles such as lipoproteins, viruses, or other colloidal size particle, the instant invention provides still another parameter, the time of passage through the pore which permits the particle's mobility to be determined. The ability to obtain this latter parameter represents a significant advance over prior art particle analyzers in that it is now possible to categorize a sample of particles according to number, size and mobility. By using this additional parameter, it is now possible to distinguish between two different types of particles of the same size. This latter feature results primarily from the difference in net electric charge which particles tend to acquire when suspended in a liquid and which is related, among other things, to the surface properties of the particular particle. Since the net electric charge of a particle under the influence of an electric field is a factor in determining how rapidly the particle passes through a pore, two different particles of the same size but of a different electric charge are distinguishable. This feature can be used to a significant advantage in categorizing blood cells, for example, according to mobility where a particular mobility categorizes a blood cell having a particular characteristic.

Another important feature of the instant invention resides in the manner in which plugging or clogging of the pore is prevented or at least substantially reduced. In a preferred embodiment of the invention, a nonionic surfactant is added to the electrolyte solution to prevent agglomeration of particles at the entrance to the pore and hence prevent or substantially reduce pore plugging or clogging. In the event that the pore becomes clogged, ultrasonic agitation of the container may be employed to dislodge any particles that block the pore. A surfactant found useful for this purpose is available from Atlas Chemical Industries, Inc., Wilmington, Delaware, under the name of Tween 60. Other nonionic surfactants or even ionic surfactants could also be employed if desired and the invention is not intended to be limited solely to the aforementioned surfactant.

FIG. 4 illustrates another embodiment of the invention wherein membranes **28** and **29** are separated by a generally micro-porous material **30** such as agar, cellulose nitrate, cellulose acetate and mixtures thereof. The membranes **28** and **29** and the micro-porous material **30** have pores **31, 32** and **33** passing therethrough, respectively. The micro-porous material **30** preferably has pores substantially smaller than the particles passing through the pore **33,** i.e., approximately a quarter of the size of the particle or less. The pores **31, 32** and **33** are preferably of substantially the same diameter and are axially aligned as illustrated in FIG. 4 so as to form an extended pore of substantially uniform diameter therethrough. The function of the generally micro-porous material **30** between the two membranes **28** and **29** is to provide a region of reduced electro-osmotic effects; that is, a region wherein the motion of the electrolyte solution, caused by the movement of ions at the pore wall, generally in a direction opposite to the movement of the particle, is considerably reduced. The porous material **30** achieves this effect by reducing the number of ions at the walls of pore **33.** The time of passage through the extended pore is then controlled by electrophoretic effects rather than the difference between electro-osmotic effects and electrophoretic effects.

Still another advantage of the embodiment of the invention illustrated in FIG. 4 resides in the nature of the signal developed across the load impedance. More specifically, colloidal particles passing through the extended pore produce an output signal having amplitude peaks as the particle enters and leaves the extended pore and an amplitude minimum during the time of passage through the porous material **30.** This signal condition is illustrated graphically by curve B of FIG. 3 wherein the first amplitude peak results during passage of the particle through pore **31,** the amplitude minimum occurring during passage of the particle through pore **33** and the second amplitude maximum occurring during passage of the particle through pore **32.** The amplitude of the first peak is depicted as being less than that of the second to illustrate how a substantial difference in the length of the pores **31** and **32** affects the pulse amplitude. By using suitable apparatus well known in the art, the time of passage through the porous material **30** and hence the mobility can be readily determined. Also the size and number of particles can be readily obtained.

In practising the embodiment of the invention illustrated in FIG. 4 for categorizing blood cells, for example, it is preferable that the pore diameter be approximately 5 to 20 microns; however, in categorizing other particles, the pore may be as small as one micron or as large as 50 microns.

The following examples are set forth to exemplify the practise of this invention. These examples include specific values of the parameters involved so that the invention may be practised by those skilled in the art. It is noted, however, that these examples are provided for purposes of illustration only and are not to be construed in a limiting sense.

EXAMPLE 1

A 10 micron-thick strip of polycarbonate resin polymer sold under the trademark LEXAN by the General Electric Company is irradiated with fission fragments and etched in NaOH to produce pores through the strip. The individual pores may vary, for example, from about 0.01 microns in diameter to approximately 10 microns in diameter. A small segment having a pore of the desired diameter, for example, 0.45 microns, is cut from the strip and mounted over the aperture **14** of the partition **13** with an epoxy cement. By the use of a steromicroscope, any undesired pores in the membrane are coated with the epoxy cement so that only a single pore exists in the membrane. The pore length may be reduced from 10 microns (the thickness of the membrane) to approximately 3 microns by stripping off several layers of the membrane material. By reducing the length of the pore, the intrinsic noise level is significantly reduced. The partition **13** is positioned within

the container **10** as illustrated in FIG. **1** and an electrolyte solution comprising 0.1 normal of potassium chloride is added to the container. Before adding any colloidal particles to the electrolyte solution, the resistance of the membrane is determined by using equation (1). If ρ is 75 ohm-cm., L is 3 microns and D is 0.45 microns, then R_p is about 15 megohms. The switch **25** is then closed and the current is found to be 0.236×10^{-7} ampers. Using Ohm's Law, the voltage gradient or field strength is about 1120 volts/centimeter. The particles to be counted, sized and categorized are then added to the electrolyte solution in chamber **11** and permitted to pass through the pore **16.** Upon passage therethrough, the current flow through the electrical circuit **22** is altered by an amount proportional to the change in resistance of the pore due to the presence of a particle passing therethrough. The change in current through the load impedance **26** causes a change in the voltage drop thereacross and hence enables the change in resistance of the pore **16** to be determined. From equation (2), the diameter of the particle can readily be computed. For example, if the change in resistance is 8.0 megohms and the resistivity of the electrolyte solution is 75 ohm centimeters, then the diameter of the particle, using equation (2) above, is equal to 0.18 microns. The time of passage through the pore can readily be ascertained from the voltage pulse developed across the load impedance **26.** This may be conveniently accomplished by differentiating the pulse and by appropriate counting means, measuring the time interval between the differentiated signals. For the above mentioned particle, the time of passage through the pore was found to be 18 milliseconds. The effective mobility (velocity of the particle divided by the electric field strength) is then equal to 1.64×10^{-5} cm²/volt-seconds.

EXAMPLE 2

Using the same apparatus as described above in Example 1, the voltage across the electrodes **20** and **21** is adjusted to yield a current flow through the circuit of 0.920×10^{-7} amperes. Particles of a different size are added to the chamber **11** and permitted to flow through the pore **16.** From the voltage pulse obtained across the load impedance, the change in resistance of the pore is computed and using equation (2), the diameter of the particle is determined. In this case, the particle is found to have a diameter of 0.091 microns which passes through the pore in approximately 6 milliseconds. The time of passage of this particle through the pore is approximately one-third of that for the previous particle which corresponds approximately to the increased voltage across the electrodes **20** and **21.**

EXAMPLE 3

An extended pore having a configuration substantially similar to that illustrated in FIG. **4** with a pore diameter of 15 microns and an overall length of 65 microns is positioned within the container **10.** The extended pore comprises a membrane **28** of 10 microns, a porous material **30** of 25 microns and a membrane **29** of 30 microns. From equation (1), the resistance of the pore is determined to be approximately 0.275 megohms in an electrolyte solution having a resistivity of 75 ohm-centimeters. Particles of an unknown size are added to the electrolyte solution and permitted to pass through the extended pore. A change in resistance of 4100 ohms due to the presence of a particle in the pore is noted and using equation (2), the diameter of the particle is determined to be 6.0 microns. With a voltage gradient of 1200 volts/centimeter across membranes **28** and **29,** the time of passage through the extended pore is found to be 160 milliseconds, 20 milliseconds through pore **31,** 80 milliseconds through pore **33** and 60 milliseconds through pore **32.** The time of passage through the central region **30** is measure of the mobility of the particle.

While we have shown and described several embodiments of our invention, it will be apparent to those skilled in the art that many changes and modifications may be made without departing from our invention in its broader aspects. For example, although an embodiment of the invention has been illustrated as employing a constant voltage source with means for sensing the change in current as an indication of the passage of a particle through the pore, it is to be understood that a constant current source could likewise be employed and the change in voltage could be used instead of the change in current. Therefore, the appended claims are intended to cover all such changes and modifications as fall within the true spirit and scope of our invention.

What we claim as new and desire to secure by Letters Patent of the United States is:

1. An apparatus for analyzing small particles comprising:

a container having a partition separating said container into two chambers, each containing an electrolyte solution, said chambers in communication with each other through a pore in a pair of membranes separated by a microporous material, said pore passing through said membrane and said microporous material;

a pair of electrodes, one positioned in each chamber;

electrical circuit means connected to said electrodes to electrophoretically drive particles through said pore; and

electric indicating means for indicating the passage of a particle through said pore.

2. The apparatus of claim **1** wherein said pore is of a diameter in the range of 1 to 50 microns.

3. A method of analyzing small particles comprising the steps of:

electrophoretically driving a solution-suspended particle through a pore in a pair of membranes separated by a microporous material, said pore having a substantially uniform diameter of between approximately 0.01 and 50 microns; and electrically sensing the time of passage of said article through said pore so that the mobility of said particle can be determined.

4. The method of claim **3** further comprising the steps of:

counting and sizing particles passing through said pore.

5. The method of claim **4** further comprising the step of:

categorizing particles passing through said pore by number, size and time of passage through said pore.

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