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DESCRIPTION

MIXING APPARATUS AND METHOD OF MIXING

The present invention relates to an improved mixing apparatus and a method of mixing.

More particularly it relates to an improved apparatus, instrument and device for conducting an assay and the assay methodology.

In a particularly preferred embodiment it relates to a device suitable for use in assaying analyses, for example glaciated proteins, in samples such as, for example, blood.

A person skilled in the art will however appreciate that the principle behind the invention can be applied to solve a mixing problem in a number of different apparatus, instruments or devices.

The applicant has developed an apparatus, instrument and device for conducting an assay as disclosed in PCT/GB98/03586. The apparatus comprises a first inlet, a second inlet, and an inlet port, the inlet port being moveable relative to each of said first and second inlets such that the port can be brought into liquid communication with each inlet in turn as required, the inlet port accommodating a filter means and/or a binder retaining means.

In the course of conducting an assay to, for example, determine the presence or absence of one or more analyses in a sample, the sample is separated into a first component fraction and a second component fraction,

the second component fraction being obtained by eluting a component "held" on the binder retaining means from the binder retaining means.

The applicant has determined that the elution step in which the elutant fills the second inlet under gravity, gives a non-homogenous sample (due to the formation of an elution gradient in the second inlet) which results in inaccurate readings when the sample is "read" in a measuring instrument, such as, for example, an instrument comprising a microprocessor operable via a keypad, one or more light emitters and one or more light detectors, a display and driver, an analogue to digital convertor and means for connecting the instrument to a power source.

It is an aim of the present invention to provide a simple method for making a gravity fed fraction homogenous and more particularly to provide a modified apparatus, instrument and/or device capable of performing such a method.

In accordance with a first aspect of the present invention there is provided an apparatus comprising an inlet port movable relative to each of first and second inlets which are or lead to first and second collection chambers, said inlet port being a funnel and accommodating a filter means or binder retaining means, the apparatus incorporating one or a plurality of means for breaking the surface tension of a drop to ensure it leaves the inlet port and enters the first or second inlet, characterized in that the apparatus further comprises a paddle for mixing a sample in a chamber said paddle comprising a magnetic or piezoelectric material such that the paddle is capable of undergoing a reciprocating motion.

It is another and independent aim of the present invention to provide an apparatus, instrument or device, capable of mixing a sample for an assay in which an

analyte is detected by a spectrophotometric means and/or a component for use in achieving this aim.

The paddle may comprise a liquid moving surface and means for supporting said paddle in or over a chamber such that the paddle can undergo a reciprocating motion in the chamber.

Preferably the means for supporting the paddle in or over the chamber comprises a pair of arms extending from the liquid moving surface.

Preferably the paddle is T shaped.

More preferably the fluid moving surface has an opening formed therein through which a light beam can pass.

In one embodiment, the paddle comprises a magnetic material and is caused to undergo a reciprocating motion by an electromagnetic means such as a solenoid.

In another embodiment, the paddle comprises a piezoelectric material and is caused to undergo a reciprocating motion using a localised current.

Of course other mechanisms could be used to effect a reciprocating motion.

Preferably at least one chamber comprises a base with sides extending therefrom to define a chamber, said sides comprising means, for example a pair of slots, which support the paddle.

Preferably the chamber is an optical chamber.

More preferably the chamber is part of a carousel or cassette.

According to yet a still further aspect of the present invention there is provided an instrument adapted to receive an apparatus as described above, said instrument

comprising means for causing said paddle to undergo a reciprocating motion in the container.

Preferably said means for causing said paddle to undergo a reciprocating motion is an electromagnetic means, for example a solenoid.

According to yet a still further aspect of the present invention there is provided a device comprising a reading instrument comprising means for driving a paddle in a reciprocating manner in an apparatus comprising an optical chamber.

An example of an apparatus and instrument which can be adapted in accordance with the present invention are described in International application PCT/GB98/03586.

The apparatus and instrument described in PCT/GB98/03586 are susceptible to a number of other problems common to devices which are used in assays. Thus, a separate and unrelated problem with a device of the general type described in PCT/GB98/03586, namely one in which a sample or samples are presented to an instrument for reading, is one of accurately positioning the sample relative to, for example, one or more of the light

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emitters and one or more light detectors which make up the reading means if reading errors are to be avoided or at least minimized.

Thus it is an independent aim of the present invention to provide a device which enables accurate readings to be taken.

According to this independent aspect of the present invention there is provided a device, comprising an instrument for reading one or more samples, and an apparatus for presenting the one or more samples to the instrument, wherein the positioning of the one or more samples into a reading position is achieved using two phased recognition.

The two phased recognition preferably utilises at least two independent micro switches.

A first switch informs the instrument that the apparatus is within range and a second switch confirms precise alignment.

A first micro switch on the instrument is activated by an "element" on the apparatus and this constitutes the first phase of detection. Preferably the element on the apparatus is a projecting member which depresses a board mounted micro-switch via a rocker arm assembly. The rocker arm actuation overcomes any error in the horizontal location of the switch on the circuit board.

A second switch on the instrument serves as the "fine tune" and is activated when the instrument reaches a precise (as opposed to general) location on the instrument.

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In one embodiment the two members of the switch are a notch in the outermost wall of the apparatus, more particularly a carousel or cassette type apparatus, and a resilient member or arm on the instrument. When the carousel or cassette type apparatus moves into position the resilient member or arm moves from a position in which the member is biased to its unbiased position thereby deactivating the switch.

This two stage recognition makes assembly easier and increases the robustness of operation. It also improves the ease of use.

In the case of a carousel device of the type disclosed in PCT/GB98/03586, it is preferred that there are a plurality of such switches. More preferably there are four such switches located 90° apart.

A separate and unrelated problem with a device of the general type described in PCT/GB98/03586 is how to achieve good readings when quantifying two different fractions.

For example in the case of diabetes management it is desirable to determine the percentage of blood haemoglobin (Hb) that is glaciated. This means two assay results need to be obtained and a comparison made between them. For example between glaciated and non glaciated haemoglobin.

Traditionally analyses are measured at a peak frequency. In the case of glaciated proteins containing haem pigment this peak frequency is around 405nm. The applicant has determined that there are significant advantages

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to be gained by making the measurements off peak, and in the case of glaciated haemoglobin protein, at between 415-460nm, more particularly still at about 440nm. This frequency range corresponds to be shoulder of the absorbance verses wavelength graph for haemoglobin. The 440 nm figure is the preferred wavelength. This extends the linear response to cover a wider and hence more useful range of haemoglobin concentrations.

According to this independent aspect of the present invention there is provided a method for determining the % glycation of blood comprising separating a blood sample into a first component fraction containing one or more non glaciated proteins, and a second component containing the one or more glaciated proteins, and detecting/quantifying the analyte by spectrophotometric means at between 405 nm and 460 nm, more preferably at about 440nm.

The "off peak" measurement avoids complicated calibration procedures, both in production and on-going in the field. It is essential for the performance of an instrument and when comparing tests between instruments that there is a linear response between measurement and concentration of absorbing substance. It is equally important that the range of linear response is wide enough so that measurement of, for example, both glaciated and unglycated fractions can be made on a linear portion of a response curve. The reason for this is that the slope of the linear response will vary for a number of reasons from instrument to instrument. Also the

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slope of the linear response will also vary within an instrument as a function of temperature or other environmental factors. However, the nature of the calculation of % glycation is such that, within a given instrument, the slope of the response cancels out. Variations in slope do not therefore effect the result either within an instrument or between instruments, as long as significant change does not occur over the period of an assay. Any remaining variation between instruments can therefore be equalised using a calibrated offset established during initial set up at manufacture.

The use of a narrow band of wavelength produces a linear response but as this nears the absorbance maximum of haemoglobin the range of response is reduced. This is because at this point the system is at its maximum sensitivity. The selection of suitable band pass filters away from the absorbance maximum de-sensitises the system and extends the working range of the response allowing the elimination of slope factors described above. The reduced sensitivity is then offset by achieving a high signal to noise ratio on the detector electronics.

A separate and unrelated problem with a device of the general type described in PCT/GB98/03586 is ensuring accuracy of readings and keeping critical values (CV[®]) to a minimum.

The applicant has demonstrated that a major factor effecting critical values is ensuring all of the sample is collected for measuring. Thus where small volumes are measured as much as 8% of the total volume can be lost

in a single drop.

A separate and unrelated problem with a device of the general type described in PCT/GB98/03586 is the problem of ensuring the apparatus is firmly held in position in the apparatus when readings are to be taken.

The Applicant has resolved this problem by careful design of the apparatus and instrument.

Thus in one embodiment the carousel apparatus comprises a tapered circumferential ring and the instrument comprises spring clips which pull the carousel downwards preventing wobble.

The main invention and various independent aspects of the invention will now be described, by way of example only with reference to a device of the general type described in PCT/GB98/03586 and a method of assaying glaciated and non glaciated haemoglobin fractions.

FIG. 1 is a perspective view of an apparatus according to one aspect of the invention;

FIG. 2 is a partial sectional view of the Fig. 1 apparatus;

FIG. 3 is a perspective view of the base portion of the apparatus of FIG. 2 showing the paddle of the invention; and

FIG. 4 is a perspective view of an instrument for use with an apparatus as illustrated in FIGS 1 to 3.

Referring to Figs. 1 and 2 the carousel apparatus 31 comprises a base section 2 of clear plastics (shown in detail in Fig. 3), a top portion 6 and a funnel portion 32. The

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funnel portion 32 is made of a hydrophobic plastics and has a relatively large aperture to simplify emptying of reagents therein.

It has an outlet 34 which directs the liquid into the optical chambers 3 and 5 when the apparatus is rotated in an instrument. The outlet 34 includes a frit (not shown) which frit serves to retain particles such as, for example, an amino phenyl boronate agarose affinity matrix. The funnel 32 which serves as an inlet port has an annular rim 36 with a recessed portion 38. The rim

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36 partially overlies apertures 40, 42 and 44 formed in the top portion 6 of the apparatus such that tubes vertically disposed in the apparatus cannot pass through the respective apertures until the apertures are aligned with the recessed portion 38 of the annular rim. Projecting from the underside of the funnel is a stem 48 with a female mating member via which the apparatus 31 is connected to the instrument 24 which has a male member 50 adapted to engage it. The male member 50 holds the funnel in a fixed position relative to the instrument 24 such that the base portion 2 and top portion 6 of the apparatus 31 which together form a carousel rotate around the funnel, the annular rim 36 of the funnel serving as a guide means.

The base portion 6 of the apparatus is made of a clear plastics, is generally annular in shape and is divided into a plurality of compartments. As can be seen from Fig. 3 there are two optical chambers 3 and 5, a third chamber 4, for receiving waste from a wash step, which third chamber is disposed between optical chambers 3 and 5, and three additional chambers 40', 42' and 44' each housing a reagent tube. These chambers 40', 42' and 44', which are disposed below apertures 40, 42 and 44 in the top portion 6 of the apparatus 31, are arranged so that the reagent tubes are presented to the user when the carousel is in the appropriate position in use. The optical chambers have a curved outer wall 52 and a curved inner wall 54 of optical quality, which help focus light from the LED's of the instrument 24 through the sample in the chamber to photodiodes at the other side thereof.

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Each optical chamber 3, 5 can be brought into liquid communication with the outlet 34 of the funnel inlet port 9. Alternatively, the optical chambers can be recessed. Extending outwardly from the outermost wall 56 of the base portion 2 is a guide member 58 which sits within a circumferential channel member 60 formed on the outermost wall 62 of the annular recess 64 of the instrument 24. A communicating channel 66 which extends from the channel member 60 in outermost wall 62 to the top face 68 of the instrument 24 allows the guide member 58 to be inserted into the channel member 60 when the apparatus 31 is connected to the instrument 24.

A projecting member or tab 70 on the knurled edge 72 of the top portion 6 acts as an indicator means, denoting the position for locating the apparatus on the instrument and serves to assist in the turning of the apparatus.

The base portion 2 is connected to the top portion and the funnel portion sits in a channel 76 formed by a step on the top surface 78 of the top portion 6.

The instrument illustrated in Fig. 4 has been designed for use with a basic apparatus as herein before described. The instrument is provided with a power management and monitoring circuit so that the instrument can be connected to, for example, an external dc supply or a car battery. Additionally, the instrument is provided with a communication system such as, for example, a RS232 thereby providing means for sending and receiving

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instructions and down loading data.

Significantly, the means for receiving the apparatus is an annular recess 64 in the instrument which is defined by a floor, an outermost sidewall 62 and an innermost sidewall 80.

The floor of the annular recess comprises a ramp 82 on a part thereof. Within the outermost sidewall 62 of the annular recess is a channel member 60 and extending therefrom to the top surface a connecting channel 66.

In use the basic apparatus is inserted into the annular recess 60 by aligning guide member 58 of the apparatus with connecting channel 66 so that the apparatus is connected to male mating member 50 via its female mating member 48. The guide member 58 can thus enter channel member 60 such that it can be rotated. On rotation a first tube is directed up the ramp 82 and out of its aperture 44 since the recessed portion 38 of the annular ring 36 is aligned with the aperture. In this position the outlet 34 is in liquid communication with the first optical chamber 3 and the first step of the assay can be conducted. By turning the apparatus through a further 90° a wash solution is presented through aperture 42 for use and then on turning the apparatus through a further 90° tube 40, the eluting solution, is presented. In this manner the appropriate reagents are presented for each step of the assay process.

Having briefly described the favoured basic apparatus and instrument there follows a more detailed look at the improvements.

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When tested an apparatus as described in PCT/GB98/03586 showed a critical value in the order of 6-7%. This was found to result primarily from an elution gradient forming when the glaciated fraction was eluted off the solid phase. In fact, it was found that the glaciated fraction was eluted off in a decreasing concentration as the elution buffer percolated into the optical chamber 5. Tests indicated that the first concentrated drops emerging from the funnel 32 collected in the corners of the optical chamber 5 and did not mix sufficiently with the more dilute drips that followed. As a result measurements taken before mechanical mixing of the solution showed poorer precision and an "off set" from those recorded post mixing.

To overcome this problem it proved necessary to introduce a mixing step.

However traditional methods proved unsuitable. Thus, for example, the apparatus could not be shaken without fear of damage to the instrument, and the use of a rotating flea or oscillating ball bearing could damage the optical chamber.

The applicant solved the problem using a paddle 100. A number of approaches were used:

Retention of a stirring device was seen as the major issue to be resolved. Attaching a stirring component to the side walls of the optical chamber was seen as a possible approach to overcome this problem. Two alternatives were investigated: In one embodiment the paddle was

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clipped over the side walls of the optical chamber and the paddle was made to vibrate in the direction of the optical axis using an electromagnet. A hole in the centre of the paddle provides a path for the light from the LED.

In another embodiment the paddle was clipped over one side of the optical chamber 5 and was made to vibrate at right angles to the optical axis away from the light path.

Both these embodiments provided adequate mixing once a resonant frequency was found by adjusting the frequency of an oscillator driving an electromagnet. Though attractive there were still a number of problems with this approach.

As the paddle must retain stiffness a significant amount of energy was required to generate the oscillation. This would have implications for any battery operated instrument.

Furthermore resonant frequencies vary from component to component and with the liquid level within a chamber. Some means of scanning the frequencies would thus be required to hit resonance and thus ensure adequate mixing. Since both components also had a 3-dimensional shape forming was required increasing costs.

An alternative approach of using a flat paddle overcame the problems associated with the oscillating approach described above.

Thus in a preferred embodiment and as illustrated in Fig.3 a metal paddle 100 was retained in grooves 101 formed by building-up the side walls

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102, 104 of the optical chamber 5. The paddle was able to reciprocate with minimal friction and could be forced to swing through the solution, along the direction of the optical axis, using an electromagnet positioned below the photodiode on the outer circumference of the platten moulding (described hereafter). A hole 106 is provided to enable the light from the LED to reach the detector.

As very little force is necessary to move the paddle, significantly less energy is required to drive the electromagnet. Experiments have shown that fewer than 10 swings of the paddle are required to produce a visually homogenous solution from a layered dye-water starting solution.

Effective retention of the paddle has been demonstrated by positioning a web (not shown) on the underside of the top moulding 6, just above the centre of the paddle.

Another improvement relates to the use of 2 micro-switches in a phased approach. This allows the precise unambiguous detection of the apparatus 31 in the instrument 24. One switch (not shown) at each of four locations is activated by a feature 58 (in this case also the guide member) on the circumference of the plastic well as it rotates. This depresses a board mounted micro-switch (not shown) via a rocker arm assembly (110, 112, 114 & 116) at each of the four operating positions (Fig. 4). The rocker arm actuation overcomes any error in the horizontal location of the switch on the circuit board (not shown). This constitutes the first phase of detection.

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The second phase of detection is provided by a micro-switch which is activated by the operation of a ratchet arm not shown with a respective notch or notches 120, 122, (only two of the four are visible) in the outermost wall 56 of the carousel. A flange 131 extending from the ratchet arm contacts a switch on the instrument. The ratchet arm is biased such that when the carousel is in one of the four operating positions it moves into a notch in the carousel, deactivating the switch but when it is not in one of these positions it is acted against by the outermost wall 56 of the carousel causing the switch to be activated. These notches are preferably shaped to allow rotation in one direction only. These switches are only de-activated when the instrument and apparatus are in an exact location. The two phased approach makes assembly easier, increases robustness of operation and improves ease of use.

Finally another improvement relates to the arrangement used to overcome a 'wobble' problem. Any movement, however small, between the carousel and instrument can alter the path of light during reading. By modifying the carousel and instrument to provide a lock facility the reading problem was overcome.

In one embodiment the carousel comprises (Fig.1) a circumferential ring 124 comprising an inclined surface 126 and a flat surface 128.

The instrument 24 which receives the carousel comprises a casing 130, a printed circuit board 132 onto which is mounted a plateen 134 and a

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hold down 136 comprising four spring clips 138, 140, 142, 144. When the carousel is inserted into the instrument, the spring clips ride up the inclined surface 126 and their claws lock against the flat surface 128.

CLAIMS

1. An apparatus comprising an inlet port movable relative to each of first and second inlets which are or lead to first and second collection chambers, said inlet port being a funnel and accommodating a filter means or binder retaining means, the apparatus incorporating one or a plurality of means for breaking the surface tension of a drop to ensure it leaves the inlet port and enters the first or second inlet, characterized in that the apparatus further comprises a paddle for mixing a sample in a chamber said paddle comprising a magnetic or piezoelectric material such that the paddle is capable of undergoing a reciprocating motion.
2. An apparatus as claimed in claim 1 in which the filter means or binder retaining means comprises a web or like member situated between the inlet port and the first and second inlets.
3. An apparatus as claimed in claim 2 wherein said web is situated across an outlet of said funnel.
4. An apparatus as claimed in claim 3 wherein said web or like member comprises a frit.
5. An apparatus as claimed in any one of claims 1 to 4 wherein the paddle comprises a liquid moving surface and means for supporting said paddle in or over a chamber such that the paddle can undergo a reciprocating motion in the chamber.
6. An apparatus as claimed in claim 5 wherein the means for supporting the paddle in or over the chamber comprises a pair of arms extending from the liquid moving surface.

7. An apparatus as claimed in any one of claims 1 to 6 wherein the paddle is T shaped.
8. An apparatus as claimed in any one of claims 1 to 7 wherein the liquid moving surface has an opening (106) formed therein through which a light beam can pass.
9. An apparatus as claimed in any of claims 1 to 8 wherein the paddle comprises a magnetic material.
10. An apparatus as claimed in any one of the preceding claims wherein at least one chamber comprises a base with sides extending therefrom to define the chamber, said sides comprising means which support the paddle.
11. An apparatus as claimed in claim 10 wherein said means which support the paddle is a pair of slots in said sides.
12. An apparatus as claimed in any one of the preceding claims wherein said chamber is an optical chamber.
13. An apparatus as claimed in any one of the preceding claims wherein said chamber is a carousel or cassette.
14. A carousel as claimed in claim 13, for use in an assay in which a sample is separated into a first component fraction and a second component fraction, which fractions are presented to an instrument, comprising a first inlet which is or leads to a first component fraction collection chamber, a second inlet which is or leads to a second component fraction collection chamber, and an inlet port accommodating a filter means or a binder retaining means, said inlet port being movable relative to

each of said first and second inlets such that the inlet port can be brought into liquid communication with each first and second inlet in turn as required.

15. An apparatus as claimed in claim 14 which is a carousel comprising a base portion having a plurality of chambers including first and second inlets, a top portion which together with the base portion forms the carousel, and a funnel portion including an inlet port, said carousel being rotatably mounted about said funnel portion.
16. An instrument adapted to receive an apparatus as claimed in any one of the preceding claims, said instrument comprising means for causing said paddle to undergo a reciprocating motion in the chamber.
17. An instrument as claimed in claim 16 in which said means for causing said paddle to undergo a reciprocating motion is an electromagnetic means.
18. An instrument as claimed in claim 17 wherein said electromagnetic means is a solenoid.
19. A device, comprising an instrument as claimed in any one of claims 16, 17 or 18 for reading one or more samples, and an apparatus as claimed in any one of claims 1 to 16 for presenting the one or more samples to the instrument, wherein the positioning of the one or more samples into a reading position is achieved using two phased recognition.
20. A device as claimed in claim 19 in which a first switch informs the instrument that the apparatus is within range and a second switch confirms precise alignment.
21. A device as claimed in claim 19 or 20 wherein a first micro switch on the

instrument is activated by an "element" on the apparatus and this constitutes the first phase of detection and a second switch on the instrument serves as the "fine tune" and is activated when the instrument reaches a precise location on the instrument.

22. A device as claimed in claim 21 wherein the "element" on the apparatus is a projecting member which depresses a board mounted micro-switch via a rocker arm assembly.

23. A device as claimed in claim 22 wherein the two members of the switch are a notch in the outermost wall of the apparatus, and a resilient member or arm on the instrument.

24. A device as claimed in claim 23 wherein the apparatus is a carousel or cassette type apparatus.

25. A device as claimed in claim 24 comprising four switches located 90° apart.

26. A method for determining the percentage glycation of blood comprising the use of a device as claimed in any one of claims 19 to 25, the method comprising the steps of:

i) separating a blood sample into a first component fraction containing one or more non glycated proteins, and a second component containing the one or more glycated proteins, and

ii) detecting/quantifying glycated haemoglobin by spectrophotometric means at between 405 nm and 460 nm.

27. A method as claimed in claim 26 wherein the detection/quantification of glycated haemoglobin is measured at about 440nm.