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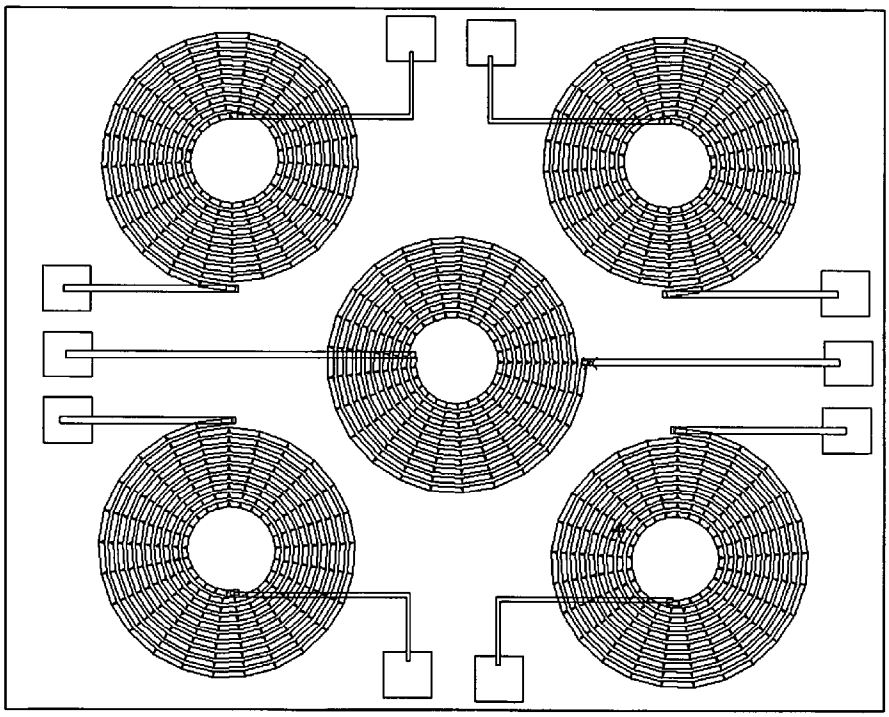

(57) Abstract: A magnetic particle detector system comprises a plurality of reaction surfaces; a corresponding plurality of electrical coils, each located adjacent a respective reaction surface; and a measuring system for measuring the effect of magnetic particles bound to a reaction surface on the inductance of the associated electrical coil.

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MAGNETIC PARTICLE DETECTOR SYSTEM

The invention relates to a magnetic particle detector system and a method for using such a system. The system is particularly useful in connection with binding assays such as immunoassays but can be used in other applications involving the binding of magnetic particles to a reaction surface.

Binding assays use complementary partners in such a way that one of the partners in a sample can be quantified. Typical examples of binding partners include: any antibody and corresponding antigen; hormone and hormone receptor; hormone and hormone binding protein; drug and drug receptor; enzyme and cofactor; chelating or complex-forming agent and an ion. One of the most common forms of binding assay is the immunoassay where the binding partners are antibodies and the targets are antigens. Immunoassays make use of the highly specific and sensitive interactions between antigens and their antibodies. Over the last 30 years they have found applications in many diverse areas including clinical chemistry and environmental monitoring. The high specificity and sensitivity arises from the nature of the interactions between the antigens, which are usually the analyte molecules, and their antibodies.

With a few exceptions (such as techniques based on surface plasmon resonance, vibrating devices, etc.), a label, reporter or marker must be added if the interactions between the antibodies and the antigens are to be quantified. Several different types of labels have been used in immunoassays. Popular labels currently used include radioisotopes, fluorescent and chemiluminescent molecules, enzymes, gold particles and coloured latex beads. Lateral flow or immunochromatographic assays are relatively simple immunoassay techniques using coloured latex beads and an example of which is the very successful Clear Blue one-step pregnancy test originally developed by Unilever. It is also an example of a device that with no

sample preparation can produce the result of an analysis in a few minutes without the intervention of a skilled operator.

There is a requirement for more such devices for use in the field in environmental monitoring or for rapid diagnostics such as point-of-care patient and veterinary testing. However, simple lateral flow devices are restricted to applications where it is only necessary to determine whether the concentration of the analyte is above or below a threshold value. If more quantitative measurements are required then elaborate measuring equipment has to be used, usually with different markers, and this adds considerably to the complexity of the technique. Techniques, such as the enzyme-linked immunosorbent assay cannot be readily adapted into rapid, portable, systems as they involve many time-consuming steps (sample separation, washing and incubation) and methods using luminescent labels need optically pure test solutions to reduce light absorption, scattering and fluorescence quenching effects. Washing steps are necessary to remove excess, unreacted (free) label and other unwanted components in the sample (free proteins, excess antibody, etc.) that are likely to interfere in the determination of the amount of bound label in the immunoassay.

Coated paramagnetic particles, PMPs, are currently used in the purification and isolation of antibodies, antigens and other proteins and more recently in immunoassays where they serve to isolate the target antigen within the interacting molecules. PMPs are available in a range of different diameters (typically, 0.1 to 20μm) from several different suppliers. They have a paramagnetic core (usually iron oxide) with a suitable coating to which capture antibody molecules are attached. In a typical automated system, an excess of the coated PMPs is introduced into a test solution containing a mixture of the target antigens (analyte) and other species. Eventually, the antigens become attached to the antibodies on the PMPs.

At this, or a later stage, an excess of the appropriately labelled secondary antibody (detecting antibody) is introduced into the test solution, which then reacts with the target antigen (analyte) on the PMPs. The PMPs with their attachments can be easily drawn to one side of the reaction vessel and held there by an external magnet whilst the remaining test solution is flushed away and replaced with clean buffer solution. The PMPs are re-suspended in the buffer solution and the quantity of bound label determined using a technique appropriate for the label. Although the PMPs help to simplify the extraction and washing processes, several steps might still be required, which add to the total time for a complete assay. Another problem is that expensive equipment is usually required to determine the amount of bound label (such as photon-counting equipment for luminescent labels, radiation-counting equipment for radioactive labels, etc.). A magneto-immunoassay system uses the PMPs as the labels, so no additional label is required.

A simple magnetometer to determine the quantity of bound PMP labels is described in EP-A-1146347. The immobilization of the magnetic particle is through, on the one hand, the interaction of a binding partner attached to the reactive surface adjacent to a detecting coil and a complementary binding partner (the target analyte) which is introduced into the system in the sample being analysed, and, on the other hand, the target analyte and another complementary binding partner attached to the particle. This assay format is called a sandwich assay and has a dose response with a positive slope. Alternatively, in a "displacement assay", suitably coated paramagnetic particles are bound to a reaction site. On the addition of sample containing the target analyte, paramagnetic particles are "displaced" from the reaction site due to competitive interaction between the target analyte and analyte immobilized on the paramagnetic particles. This will result in a loss on paramagnetic material from the

reaction site. This assay format has a dose response with a negative slope. In this mode the particles may be immobilised on the reactive surface prior to sample being added to the liquid or the particles may be added with the sample or following the sample. Alternatively, in a "competitive assay", a binding partner is attached to the reaction site. A first complementary binding partner (the target analyte), which is introduced to the system in the sample, competes with a second complementary binding partner (the "competitor") to bind to the reaction site. The second complementary binding partner is attached to the magnetic particle. The greater the concentration of target analyte, the fewer magnetic particles will succeed in binding to the reaction site. This form of assay therefore has a dose response with a negative slope.

Various other forms of "competitive assay" exist. For example, the binding partner may be provided on the magnetic particle and the competitor (the second complementary binding partner) on the reaction site. The target analyte, introduced in the sample, competes with the competitor to bind to the magnetic particle. The greater the concentration of target analyte, the fewer magnetic particles will bind to the competitor on the reaction site. Therefore this form of assay also has a negative dose response.

Typically, binding partners are attached to the particle and reactive surface through electrostatic and other non-covalent bonds; covalent bonds through reactive groups on the binding partners and particle or reactive surface; through an intermediary biological molecule attached to the surface as above; or through any process of molecular imprinting or melding. To prevent non-specific binding of either binding partner to the reactive surface or particle during the analytical reaction, a blocking agent is used to bind to vacant binding sites on the reactive surface or particle. Blocking agents used do not bind with either of the binding partners and are typically

made from inert proteins or protein hydrolysates, ionic or non-ionic detergents, simple or complex sugars, inert polymers.

The reaction takes place in a liquid designed to promote the interaction of the binding partners. This liquid may contain buffer salts, poly-ionic molecules, polymers, simple or complex sugars and proteins. Binding partners may include any substance of biological or chemical origin such as proteins, nucleic acids, carbohydrates, lipids, drugs, chelating agents, spores, micro-organisms or cells isolated from tissue culture or blood sample or tissue biopsies. "Chemical" substances include non-biological substances such as polymers. Typical examples of binding partners include: any antibody and corresponding antigen, hormone and hormone receptor, hormone and hormone binding protein, drug and drug receptor, enzyme and cofactor, transcription factor and DNA, subunits of a protein complex such as G-proteins, any signalling or transport protein and its control element, lectins and glycoproteins, lectins and carbohydrate moities, receptor protein and lipoprotein, lipid and lipoprotein, DNA and DNA, DNA and RNA, RNA and RNA, PNA and PNA, PNA and DNA, PNA and RNA, cell membrane proteins and virus, cell membrane proteins and spores, cell membrane proteins and bacteria, any cell to cell interaction through any cell surface binding protein such as MHC II and CD4.

In a preferred embodiment, a sandwich immunoassay system is used and magnetic labels are immobilized onto a reaction surface and a magnetometer then determines the quantity of immobilised magnetic labels.

The basic magnetic particle detection system consists of a coil (inductor), which forms part of a resonant network, a detector circuit and a test strip or test vessel. During operation the coil is located close to a reaction surface either on the test strip or in the test vessel. Magnetic particles bound on the reaction surface influence the high frequency magnetic field produced by the

coil and cause a change in the inductance of the coil. The magnetic particles may contain paramagnetic, ferrimagnetic, anti-ferromagnetic or ferromagnetic materials. This change in inductance is a function of the quantity of magnetic particles immobilised on the surface. The change in the inductance of the coil is detected by a change in the resonant frequency of an inductance and capacitance (LC) network, of which the coil forms the inductive component and this is used to determine a measure of the number of particles attached to the reaction surface.

This detection system works well but is limited in that it is only able to detect a single target analyte at any one time.

In accordance with a first aspect of the present invention, a magnetic particle detector system comprises a plurality of reaction surfaces; a corresponding plurality of electrical coils, each located adjacent a respective reaction surface; and a measuring system for measuring the effect of magnetic particles bound to a reaction surface on the inductance of the associated electrical coil.

We have developed a multi-analyte system which is able simultaneously to detect more than one analyte within a sample by providing a plurality of corresponding reaction surfaces and associated electrical coils.

We have found that it is possible to design coils which respond to magnetic particles close to the coil but not to particles that are located away from the relevant reaction surface. Thus, unreacted particles that are still in suspension in the reaction solution do not influence measurements of the bound particles and they do not have to be removed before measurements are made.

The reaction surfaces could be defined by distinct structures or by different regions of a single reaction surface.

An important application of the invention is in a method of performing a binding assay, for example an immunoassay, using a device according to the first aspect

of the invention, the method comprising providing different binding partners at the reaction sites; supplying a sample to the reaction vessel or test strip; supplying coated magnetic particles which attach to the binding partners via respective target analytes in the sample whereby the magnetic particles are caused to attach to the reaction sites in the presence of the target analytes in the sample; and monitoring the affect of magnetic particles bound to the reaction surfaces on the inductance of the coils to detect the quantity of magnetic particles attached to the reaction sites.

In an alternative method, the coated magnetic particles are attached directly to the immobilized binding partners at the reaction sites, and the addition of the target analytes in a sample causes the magnetic particles to detach from the reaction sites, due to preferential binding (higher affinity) for the analytes. Monitoring the affect of the magnetic particles, as indicated above, can be used to determine the amount of analyte bound at the reaction sites.

Further alternatively, the method could comprise providing different binding partners at the reaction sites; supplying a sample to the reaction vessel or test strip, the sample including at least one target analyte which is complementary to the binding partners at one of the reaction sites; supplying at least one competitor to which magnetic particles are attached, the or each competitor being complementary to the binding partners at one of the reaction sites, whereby the or each target analyte inhibits the binding of its respective competitor and attached magnetic particles to the respective reaction site; and monitoring the effect of magnetic particles bound to the reaction sites via the or each competitor on the inductance of the coils to detect the quantity of magnetic particles attached to the reaction sites.

In a further example, the method could comprise providing different competitors at the reaction sites;

supplying a sample to the reaction vessel or test strip, the sample including at least one target analyte; supplying coated magnetic particles which are provided with binding partners which are complementary to at least one target analyte and to its respective competitor, whereby the or each target analyte inhibits the binding of the magnetic particles to the respective reaction site via its respective competitor; and monitoring the effect of magnetic particles bound to the reaction sites via the or each competitor on the inductance of the coils to detect the quantity of magnetic particles attached to the reaction sites.

In this case the magnetic particles may each be provided with a single binding partner which binds to only one of the competitors (and hence reaction sites). More than one such "type" of coated magnetic particle may be supplied, for instance, one "type" adapted to bind to each competitor. Alternatively, the coated magnetic particles may be provided with more than one binding partner or a species which enable binding to several (or all) of the different competitors.

The sample can comprise any biological or chemical (including non-biological) substances.

In some examples, the sample comprises one of whole blood, plasma, serum and urine. Assays can be performed to detect environmental analytes such as toxins or pollutants in a range of samples such as water samples from rivers, reservoirs, lakes, sewage outlets, farm run-offs, soil extracts and plant extracts.

Some examples of magnetic particle detector systems and their use in binding assays will now be described with reference to the accompanying drawings, in which:-

Figure 1 illustrates an arrangement of five coils;

Figures 2a, 2b and 2c illustrate three examples of a phase locked loop circuit;

Figure 3 illustrates a typical response of a magnetometer to the presence of magnetic particles;

Figure 4 illustrates a first example of a system according to the invention;

Figure 5 illustrates a second example of a system according to the invention;

Figure 6 illustrates a third example of a system according to the invention;

Figure 7 is a schematic cross-section through an example of a system according to the invention;

Figure 7a depicts an example of a printed circuit board suitable for use in a system according to the invention;

Figure 8 illustrates the system of Figure 7 in conjunction with an electromagnet;

Figure 9 illustrates a sandwich assay schematically;

Figure 10 illustrates a typical response from a sensing coil when a sample is added to the reaction vessel;

Figure 11 illustrates two dose response curves measured simultaneously using a dual coil substrate;

Figure 12 is a typical recording taken with the application of a steady (non-pulsed) magnetic field;

Figure 13 illustrates a dose-response curve for CRP;

Figure 14 illustrates the theoretical decrease in relative intensity of magnetic field with distance from the coil; and,

Figure 15 is a schematic diagram illustrating how bound magnetic particles produce a stronger signal than unbound particles.

Magnetic particle detector systems according to the invention comprise three key elements namely the resonant coils, the measuring system or detector circuit, and the test strip or test/reaction vessel. These will be described in turn.

1. Resonant Coils

A variety of coil designs including helical, conical or flat-plate spiral can be used. Typically, a flat-plate spiral design is used because the electromagnetic field

distribution is concentrated close to the surface of the coil so that when the coil is placed adjacent to the reaction surface(s) the electromagnetic field does not penetrate far beyond the reaction surface(s). The coils can be either a single layer flat coil or a multi-layer flat coil. Preferably the coils will be deposited onto a substrate made from an electrically insulating material. Suitable substrate materials include, a polymer, an amorphous material (such as glass, ceramic, etc), a semiconductor (such as silicon, germanium, gallium arsenide, etc), a crystalline material (such as mica, graphite, diamond, etc), or a composite (such as glass or carbon fibre board etc). The substrate material may be flexible or rigid. In a preferred embodiment the substrate material is ceramic. The coils can be fabricated from any conducting or semiconducting material or mixtures of materials. Examples would be obvious to an expert. In a preferred embodiment gold is used as the major element in the coil fabrication. In an alternative embodiment doped silicon is used as the coil material. The coils can be deposited by a variety of means e.g. electroplating, vacuum deposition, sputtering, screen printing, ion implantation, diffusion or other well-established techniques. In one preferred embodiment thick film printing technology is used. This technique provides a low cost technique for film deposition. Typically, gold ink (Agmet ESL part no. 8880-H) is used as the coil material. The diameter of the coils can vary from sub-micrometre sized coils to more than 1cm depending on the technology used and the application. The number of turns on the coil determines the inductance of the coil and is chosen to be compatible with the detector circuitry.

In a multi-analyte system there is a different binding and affinity partner for each analyte on a reaction surface. Several coils are used for measurement of a number of analytes. The arrangement of the coils is such that at least one can be positioned close to each reaction

surface. In a preferred embodiment, five coils are arranged on a square substrate. The arrangement is illustrated in Figure 1. In this figure, each coil has five turns and the track width is 100μm with 75μm spacing. The thickness of the track is 8μm. The inner radius of the coil is 500μm. In this example the coils are of identical design, but this is not necessarily the case. During operation the coils on the substrate are arranged relative to the reaction surface, such that the electromagnetic field associated with each coil covers all or a portion of the reaction surface. In some cases, the coils could be on a support separate from a surface defining the reaction surface and brought adjacent the reaction surface.

Typically, at least one of the coils will be selected as a reference coil. In a preferred embodiment, the reaction surface corresponding to the reference coil is identical to the other surfaces in most respects except that it contains no capture molecules, in which case no magnetic particles can be captured and they might, or might not, contain a pre-determined number of magnetic particles. In a further embodiment, the reaction surface or surfaces contains capture molecules of a certain type so that a predetermined number of magnetic particles always binds regardless of the concentration of the analyte. The resonant frequency of the reference coil(s) can then be compared with the resonant frequencies of the test coils. This has the benefit of reducing errors in readings caused by factors such as noise, interference and temperature variations.

2. The detector circuit

The coils are connected in parallel or in series with one or more capacitors to form a LC resonant network. The capacitor values are chosen in order to create circuits that resonate at frequencies compatible with the measurement circuit. Resonant frequency measurements can be carried out using the LC network to control the output

frequency of an oscillator such as a Colpitts, Hartley or Armstrong oscillator. In a preferred embodiment, a phase lock loop (PLL) circuit is used to determine the resonant frequency of the LC network. In an example of a PLL (Figure 2a), the detector circuitry comprises five main stages. These are a voltage controlled sine-wave oscillator (VCO) 1, a LC network 2 including the coil L and a capacitor C, a phase detector 3 and a loop filter network 4. This operates as follows. The VCO 1 drives the LC network 2. The initial frequency of the VCO 1 should closely match the resonant frequency of the LC network 2. When the frequency of the VCO is identical to that of the resonant frequency of the network, there is a zero phase difference across the resistor R. If the VCO frequency drifts from the resonant point then the phase detector 3 senses a phase difference across the resistor R and produces an error signal. The error signal is filtered and supplied to the VCO 1. This changes the oscillator frequency to again match the resonant circuit. Thus a stable resonant circuit is provided. The inductance of the resonant coil L changes when magnetic particles are immobilised on the reaction surface close to the coil. The change is dependent on the quantity of particles present. This inductance change results in a variation in the resonant frequency of the LC network 2. Consequently, as the VCO 1 and the resonant circuit frequencies are no longer identical, a phase difference is detected across the resistor R and an error voltage is supplied to the VCO to alter the output frequency appropriately. The new stable frequency produced by the VCO is the resonant frequency of the network with magnetic particles close to the coil. A typical plot of the number of magnetic particles versus decrease in resonant frequency as detected by a meter 5, is shown in Figure 3.

In another example of the phase-locked loop circuit (Figure 2(b)), the resonant frequency of a LC network 6 incorporating the coil 7 and a variable capacitor 8 is

locked onto the output frequency of a very stable sine wave oscillator 9 (such a temperature regulated crystal-controlled oscillator). The output from the oscillator 9 is fed to the LC network 6 via a resistor R as before. The values of L and C are chosen so that the resonant frequency of the LC network is close to the output frequency of the oscillator, but in this case, C is a variable voltage-controlled capacitor (varicap or varactor) 8. A phase detector circuit (PSD) 10 senses the phase of the signal across R and, as before, produces a dc-error signal, which in this case is fed via a loop filter 11 to the varactor 8. The error signal adjusts the value of C until the resonant frequency of the LC network matches exactly the output frequency of the stable oscillator. A sample containing magnetic particles brought near to coil causes the inductance of the coil to increase and the PSD circuit 10 responds by changing the error signal so that resonant frequency of the LC network again matches that of the oscillator. The magnitude of the error signal V_{DC} is related to the number of magnetic particles in the sample and is monitored by a meter (not shown).

In a simple approach, each coil can be connected to a respective circuit of the type shown in Figure 2a or Figure 2b. The simplest way of achieving this is to use individual phase-locked circuit for each coil and a common controller or monitoring system to read the output frequency of each PLL circuit via a programmable switch. Whilst this arrangement will allow rapid measurements, it uses a lot of components and is likely to be expensive.

The arrangement shown in Figure 2c locks a VCO 100 to a crystal reference signal 102. A PMP sample is applied to the low Q sense coil 104. The inductance of the sense coil, which is the tuned element of the Local Oscillator (VCO) is mixed at 106 with the crystal oscillator RF signal to produce a change in the Intermediate Frequency 108. This change is phase detected (PFD) 110 to give a DC error and applied as an automatic control signal to the VCO sense

tuned circuit variable capacitance diodes. This re-adjusts the resonance of the tuned sense circuit and keeps it locked to the reference signal. The DC error voltage in milli and micro volts is the measure of the sample.

Preferred circuits to be used in multi-coil, multi-analyte systems

In this section, we describe various circuit arrangements we have actually used for multi-coil measurements and describe other possible circuit arrangements. The controller mentioned in all these circuits could be dedicated hardware, such as a group of logic circuits, a microprocessor or a personal computer.

In a first embodiment of the application of the magnetometer in a multi-coil system having a reference coil associated with a reference reaction site and a test coil associated with a test reaction site, one phase locked loop circuit 25 is used to measure the resonant frequencies of the reference LC network and the test LC networks in sequence using a programmable radio-frequency switch 24 (Figure 4). In one arrangement, individual varactors C_R,C_S could be used with each coil 20,21 to provide resonant circuits and each capacitor (C_S and C_R) then adjusted by a common controller 22 so that immediately before adding the test sample, the resonant frequency of each LC network is identical. Alternatively, each coil 20,21 could be trimmed during manufacture to have identical electrical characteristics and then a single common capacitor (C_C) connected to the pole of the switch 24 would form a resonant circuit with a particular coil when the switch has selected that coil: in this way, the resonant frequency of each coil would be identical prior to adding the test sample. For clarity, only two coils are shown in Figure 4, although in practice any number of coils could be connected via the switch 24 to the PLL circuit 25. The controller 22 moves the switch 24 sequentially from one LC network to the next and the resonant frequency of each is measured and

stored by the controller. A disadvantage experienced with the circuit shown in Figure 4 is that the PLL circuit can take a long time to settle down after the switch has moved to a new position, which means that data are not collected fast enough to give the reliable kinetic readings using the technique described in the last section. An embodiment that reduces this problem is shown in Figure 5.

In Figure 5, one of the coils L_R and one of the capacitors C_R are used as a reference circuit 31. A PLL circuit 30 measures the resonant reference frequency (f_R) of the L_RC_R circuit 31. The test coils (L_{S1} to L_{Sn}) are connected in parallel with respective varactor capacitors (C_{S1} to C_{Sn}) and the resonant frequency of each LC circuit $32_1...32_n$ determined by individual PLL circuits (PLL_1 to PLL_n). The resonant frequency of $L_{S1}C_{S1}$ at the output of PLL_1 is fed into a mixer circuit 33_1. The reference frequency f_R is also fed into the mixer circuit 33_1 which produces a beat frequency that is equal to the difference in the resonant frequency of $L_{S1}C_{S1}$ network 32_1 and f_R. A similar arrangement of PPL and mixers $33_2...33_n$ circuits is associated with each of the other test LC circuits. The output of each mixer circuit is connected via a programmable switch 34 to a controller 35, which could be dedicated hardware or a personal computer. Before the test sample containing n analytes is added, the controller 35 adjusts the values of C_1 to C_n until there is zero beat frequency from each mixer circuit $33_1...33_n$. When the test sample has been added and the reactions proceed on the individual reaction surfaces for the analytes, the resonant frequencies of the associated LC circuits decrease, producing beat frequencies in the separate mixer circuits, which are read by the controller 35. An advantage of this circuit is that it is much faster than the one shown in Figure 4. A disadvantage is that it uses a lot of electronic components.

A potential problem when using substrates containing many closely-spaced coils is that crosstalk could occur

between the coils, leading to faulty operation of the phase detectors. One way of reducing this potential problem is to operate each coil at widely differing frequencies so that pickup by one coil of the electromagnetic radiation from another can be easily filtered out. An alternative approach is to ensure that each coil runs at exactly the same frequency. Figure 6 shows such an arrangement. A crystal-controlled oscillator 40 produces a high-purity sine wave of a fixed, stable, frequency. The oscillator could also be temperature controlled to give greater stability. This waveform is fed to all n of the LC resonant circuits $41_1 \ldots 41_n$ on the sensing device via suitable feed resistors R1 to Rn as shown. The values of L and C are chosen so that the resonant frequency of each LC network is close to the fixed frequency of the crystal-controlled oscillator 40. Each LC network $41_1 \ldots 41_n$ has a phase-sensitive detector (PSD1 to PSDn) connected across the feed resistors. The output of each PSD is filtered and then connected via a programmable switch 43 to a controller 42. The output of each PSD is a dc voltage that is read in turn by the controller 42 which also provides a measuring system. Before samples are added to the reaction vessel, the controller 42 starts by selecting the output from PSD1 and then adjusts C_{S1}, which is a varactor, until the output voltage from PSD1 is 0: at this point there is no difference in phase in the sinusoidal waveform across R1 and the resonant frequency of the $L_{S1}C_{S1}$ network exactly matches the frequency of the sine-wave from the crystal-controlled oscillator. The controller 42 now moves to each position of the switch 43 in turn and repeats the process for each LC network until the resonant frequency of all the LC networks exactly matches the output frequency of the crystal-controlled oscillator. At least one of the coils can be a reference coil. When a sample containing n analytes is added to the reaction vessel, magnetic particles become immobilised on the relevant reaction surfaces next to the coils depending on the nature of the

assay, and the resonant frequencies of the associated LC networks change accordingly. This causes the outputs of the associated PSDs to change from 0 with magnitudes that are directly related to the concentrations of the magnetic particles bound to the associated reaction layers, which in turn are related to the concentrations of the analytes. The controller 42 reads these signals sequentially via the programmable switch 43. An advantage of this circuit is that fewer components are used compared with the other circuits.

3. Test strip or test vessel

Binding reactions between binding partners where one partner is the analyte in the sample and the other partner is the capture molecule immobilised on the reaction surface take place on a substrate in a test vessel or on a test strip. The immobilised binding partners on the reaction surface will depend on the nature of the analysis and could be a multi-layer arrangement containing linker molecules to fix the capture molecules to a substrate material and blocking molecules to reduce non-specific binding. At the end of the analysis, the number of magnetic particles immobilised on a suitable reaction surface is related to the concentration of the analyte. Suitable substrate materials could be a ceramic, polymer, amorphous material (such as glass), a semiconductor (such as silicon, germanium, gallium arsenide, etc.), a crystalline material (such as mica, sapphire, diamond, etc.), or a composite (such as glass or carbon fibre board etc.) depending on the nature of the immobilised binding partner in the reaction surface. Typically the substrate material will be thin (for example 1mm). In a multi-analyte system, the reaction surfaces for each analyte may be in separate defined areas on the substrate. In a preferred embodiment, the substrate also forms a base or other wall of the test vessel 49. During operation, each sensing coil must be close to its respective analyte reaction surface. In one embodiment

(Figure 7), the coils 50,51... are mounted outside the reaction vessel 49 but positioned close to their respective analyte reaction layers 52,53, which are attached to the substrate 54 forming the wall of the reaction vessel and are inside the vessel. In this embodiment, the substrate for the coils and the substrate for the reaction surface(s) may be made from different materials. In a preferred embodiment, the reaction surfaces and the coils are both mounted on the same, thin substrate. In one version of this preferred embodiment, the reaction surfaces are deposited in defined areas on one side of the substrate and the coils 50,51 are formed on the other side directly opposite their respective reaction surfaces (Figure 7). In an alternative version of the preferred embodiment, the coils are first deposited on the substrate, covered with a thin insulating layer and then coated with a thin layer of their respective reaction surfaces.

The dimensions of the test vessel will be dependent on the volume of the sample and size of the reaction surfaces. In one example (Figure 7), the reaction surfaces are coated on one side of a substrate with the coils on the other side. The substrate 54 also forms the base of the test vessel. A typical size for this substrate would be 9mm by 9mm. The walls of the vessel are formed from a hole cut in a sheet of printed circuit board (PCB) 55: any insulating material, such as glass, mica or ceramic, is also effective for this purpose. The depth of the well is determined by the thickness of the PCB and this is typically 1 mm. In this example, the coils are deposited on the external side of the substrate. Connections between the coils and the detector circuit can be made by a variety of means including wire bonding, W strip or mechanical clips. In the arrangement shown in Figure 7 the PCB not only acts as the walls of the vessel but also as a connector as tracks are etched onto the board.

Capacitors and other components from the detection circuit can be mounted on the substrate 54 and/or the PCB

55. An example of a PCB layout is shown in Figure 7a. In this example, the walls of the reaction vessel are formed by a square hole 55' in the PCB 55. Typically, hole 55' is about 9mm by 9mm in size. A number of precautions could be taken to reduce effects that cause variations in the impedance value of the each of the resonant circuits. The tuning capacitors C_{s1} ...C_{sn} (<5% tolerance) could be positioned as close as possible to each coil on the edge of the hole 55'. The track lengths could be equalised whilst minimising crosstalk by producing tracks having "meandering" paths. Further, all the leads on the PC (including those connecting the PCB to the controller 35 and those connecting the capacitors C_{s1} ...C_{sn} to the coils) could also be designed in order to match the lengths of the tracks to one another as closely as possible. All the earth connections of the coils could be linked in a star arrangement on the underlying substrate.

Typical PCB dimensions may be about 50mm by 24mm, the hole 55' being centred across the width of the PCB 55 adjacent one end of the PCB 55.

In another embodiment, a test vessel is formed by creating a well in a sheet of material and the coils are deposited on or fabricated in one, or more, of the sides of the vessel. In a further embodiment, coils are formed on a flexible substrate material, such as a polymer. Using integrated or thick film circuit techniques, it is possible to incorporate some, or all, of the components such as the capacitors, phase-locked loop circuits, programmable switches and mixer circuits into a single device that could be fixed to the substrate along with the coils. Components could then be trimmed in the manufacturing stage and variations in coil, and other component characteristics minimised. This would improve consistency and repeatability between different coil devices and lead to a more compact, portable device.

A temperature-controlled heater could also be incorporated on the substrate by depositing a metal film

onto the substrate. A heater may be necessary in field applications when the ambient temperature is so low that it affects the chemical reactions in the vessel and, or, produces such large temperature drift in the values of the components on the substrate that they are outside their calibration range.

The Application of External Magnetic Fields to Accelerate Reactions

An advantage of magneto-immunoassays over immunoassay methods using different labels is that washing steps are not necessary because there are no significant magnetic materials in a typical sample that will interfere with the measurements. In clinical use, samples of whole blood, plasma, serum, urine, etc. may be used without further preparation. In environmental monitoring, samples taken from rivers, lakes, etc. that might be contaminated with magnetic materials can be easily cleaned prior to analysis by simply applying an external magnetic field to remove any interfering magnetic materials.

We have also found that applying an external magnetic field using permanent magnets or electromagnets can considerably reduce the total time for an assay. Figure 8 shows a typical arrangement using the multi-coil reaction vessel described earlier and an electromagnet 60.

Pulsed magnetic fields were used in the earlier experiments, and these were produced by applying a pulsed current to an electromagnet. An intense, localised, magnetic field with a steep field gradient is obtained by placing iron or steel studs 61,62 on the electromagnet and positioning then directly below each sensing coil 50,51 so that the magnetic particles are pulled towards the centre of the coils. Instead of using separate metal studs, the pole pieces of the electromagnet could be shaped accordingly.

Figure 9 shows schematically what happens when the external magnetic field pulls a coated magnetic particle,

P through the test solution containing the analyte Q towards an immobilised capture molecule, S. The capture molecules on P bind to Q to form a complex, R.

$$P + Q \underset{K_2}{\overset{K_1}{\rightleftharpoons}} R$$

K_1 and K_2 are the rate constants as shown. If there is strong affinity between the coated magnetic particle and the analyte molecules, then K_2 may be ignored. The reaction between P and Q is likely to be rapid and if the test solution containing Q is premixed with a solution containing P before the magnetic field is switched on, then the resultant solution will contain mainly the complex R. When the magnetic field is switched on, the complex R is attracted to the immobilized capture molecules, S, and a sandwich complex, T, forms with the magnetic particle immobilised in the reaction surface on the substrate.

$$R + S \underset{K_4}{\overset{K_3}{\rightleftharpoons}} T$$

Where, K_3 and K_4 are the rate constants. Again if there is a strong affinity between the analyte molecule and the immobilised capture molecule in the reaction surface, then K_4 may be ignored. The build up of the sandwich complex $[T]_t$ on the substrate with time, t, should follow a first order reaction:

$$[T]_t = [Q](1 - \exp\{-K_3 \times t\}) \quad (1)$$

Thus, $\frac{d[T]_t}{dt} = [Q] \times K_3 \exp\{-K_3 \times t\}$

and $\frac{d[T]_t}{dt}$ is a maximum when $\exp\{-K_3 \times t\} = 1$,

i.e. when $t = 0$.

The signal recorded by the sensing coil is inversely related to $[T]_t$; so the rate of fall in resonant frequency of the sensing coil just after the test sample is added to the reaction mixture is a maximum and directly related to the initial concentration [Q] of the analyte molecules.

In practice, it is often difficult to determine the initial fall in resonant frequency because of mixing effects when the different reagent and test solutions are first added. An alternative method is to wait for a certain period, t_1, after adding the solutions when the reactions have settled down and then to measure the gradient of the rate of fall in the resonant frequency over the time period, $t_2 - t_1$.

Expanding equation (1) as a power series:

$[T]_t = [Q](1 - \{1 - (K_3 \times t) + ...\})$ ignoring higher terms as $K_3 \ll 1$

$[T]_t = [Q] \times K_3 \times t$

Suppose, when $t = t_1$, $[T]_t = [T]_1$ and when $t = t_2$, $[T]_t = [T]_2$

Then,

$$\frac{[T]_2 - [T]_1}{(t_2 - t_1)} = [Q] \times K_3$$

Again, as the signal recorded by the sensing coil is inversely related to [T], the gradient of the decrease in resonant frequency over the time interval $(t_1 - t_2)$ is directly related to the initial concentration of the analyte, [Q]. Figure 10 shows a typical response from the detection coil when a test sample is analysed using the reaction vessel shown in Figure 8. Before the sample and magnetic particles are added to the reaction vessel, the resonant frequency of the detection coil drifts with temperature changes in the environment (line AB). Solutions containing the analyte, Q, and an excess of the coated magnetic particles, P, are mixed together and the

mixture added to the reaction vessel: reactions between P and Q commence and the complex R is formed. A pulsed current is now passed through the electromagnet. During the periods when the current is on, the magnet particles are pulled towards the reaction surface. Reactions occur between the magnetic particles, analyte and the capture molecules, S, resulting in the complex, T and immobilisation of the particles and producing a decrease in the resonant frequency of the sensing coil. During the period when the current is off, unbound complexes, R, and unbound particles, P, are free to move away from the reaction surface, resulting in a slight increase in the resonant frequency: particles, P, may now react with remaining un-reacted analyte molecules, Q, and complexes R may re-orientate so that they are more favourably aligned to react with the capture layer S. When the current again passes through the electromagnet, the unbound magnetic particles are pulled to the reaction surface and further reactions take place. The process continues until all of the analyte molecules have been captured in the complex T or all of the available capture molecules S have been used up (point F on Figure 10). The decrease in frequency EF is thus related to the concentration of the analyte.

However as explained earlier, the rate at which the resonant frequency of the coil falls over a particular time period after adding the sample is related to the concentration of the analyte and this is determined by measuring the gradient of line CD. This gradient can be determined in just 3 or 4 cycles of the electromagnet, which is considerably shorter than in previous methods. If this measurement is repeated with a sample containing a different concentration of the analyte, then the gradient of line CD changes accordingly. In addition, subtracting the gradient of line AB from line CD reduces errors caused by drift in the resonance coil. In multi-coil arrangements, one of the coils could be a reference coil

and drift in the resonant frequency of this coil could be used to correct for drift in the others.

An Example of the Application of the Technique: The Simultaneous Measurement of the Concentrations of troponin I (TnI) and creatine kinase MB (CKMB) in a solution containing a mixture of the two proteins.

The reaction vessel described in Figure 8 was used. The upper surface of a ceramic square substrate with two sensing coils deposited on the lower surface was prepared by first washing in 6M nitric acid followed by 2M sodium hydroxide and then de-ionised water. The upper surface was then activated by applying polymerised glutaraldehyde for 30 minutes (prepared by adding 500μl of 0.1M sodium hydroxide in 5 ml of 5% glutaraldehyde and neutralized with 500μl of 0.1M hydrochloric acid after 30 minutes at room temperature). Following activation, the surface of the ceramic substrate was washed with methanol for 1 minute. Mouse anti-human TnI or goat anti-human CKMB (Randox Laboratories Ltd., UK) was diluted 1:500 times in 0.1M bicarbonate buffer (pH 9.7) containing 2% methanol and 0.5% glutaraldehyde. Ten μl of the solution containing TnI antibodies was applied to the activated ceramic square as a spot directly above the centre of one coil on the underside and 10 μl of the solution containing the CKMB antibody was applied to the activated surface above the other coil, and allowed to incubate for 4 hours at room temperature. The surface or the chip was then washed in 50 mM phosphate-buffered saline (pH 7.4), (PBS) and blocked with 1% bovine serum albumin in PBS to reduce non-specific binding. Paramagnetic particles were prepared by coating Seradyn protein G coated particles with mouse anti-human TnI antibody or mouse anti-human CKMB antibody (Randox Laboratories Ltd.) and then diluted 1:1000 times in 0.1 M phosphate buffer (pH 8.1). Ten μl of paramagnetic particles were washed 3 times in the phosphate buffer and then mixed with 30 μl of the antibody solution and

incubated for 10 minutes at room temperature in a slowly rotating sample mixer (Dynal UK Ltd). The paramagnetic particles were then washed 3 times in phosphate buffer before being re-suspended in 20 nM dimethyl pimelimidate (Sigma-Aldich, UK) and incubated at room temperature with rotational mixing for a further 30 minutes to promote cross linking. The reaction was stopped by suspending the paramagnetic particles 50 mM Tris(hydroxymethyl)aminoethane buffer (pH 7.3). Unoccupied sites on the particles were blocked using 1% bovine serum albumin in PBS. Standards were prepared by diluting stock solutions of human TnI in serum (100 μg/ml, Randox Laboratories Ltd) and human CKMB in serum (10 μg/ml, Randox Laboratories Ltd) in 1% BSA in PBS to give a range of solutions with known concentrations.

The detector coils on the ceramic chip substrate were placed above the steel studs to focus the magnetic field to the centres of the coils allowing paramagnetic particles to be pulled on to the immobilised antibody above the coil. This enables the antigen to cross-link the antibodies on the ceramic surface and the paramagnetic particles more readily and thereby accelerate the immune-reaction as explained above. The resonant frequency of the detector coils were recorded every one second on to a PC for further data analysis.

Use of a Pulsed External Magnetic Field to Accelerate the Reactions.

Before the assay, 120 μl phosphate buffered saline was placed into the vessel and recording of the resonant frequency of the sensing coil started. This gave the background drift of the resonant frequencies of the coils. After three minutes 10 μl coated paramagnetic particles (a 1:1 mixture of the different antibody coated magnetic particles was used when 2 analytes were present) and 10 μl of the standard solution was added to the well and the electromagnet that pulled the paramagnetic particles onto the surface was switched on after a further 30 seconds. A

symmetrical pulsed waveform with a frequency of approximately 0.05 Hz (approximately 10s on and 10s off) was used to drive the power supply to the electromagnet causing the magnet field to be alternately on and off. When the electromagnet was "on", the paramagnetic particles were pulled on to the reactive surface. During the "off" phase, the unbound paramagnetic particles were free to move in the buffer. Sixty seconds (t_1) after adding the particles, the slope of the recorded signal was determined over the next 100 seconds, (t_2 - t_1). The results were calculated as explained above by plotting the difference in the slope of the recorded signal before the addition of paramagnetic particles and that recorded over the (t_2 - t_1) period. The experiment was repeated for solutions containing different amounts of TnI and CKMB. The results are plotted in Figure 11 and they clearly show that a single ceramic chip device can determine the concentrations of the two different analytes at the same time. If the ceramic substrate contained 3 coils then the third coil could be used a reference coil and used to monitor the drift in the measurements caused by temperature changes. In our previous method of measuring we used two magnets alternately, one placed below the sensing coil to pull the magnetic particles to the reaction surface and the other positioned above the reaction vessel to pull unbound particles away from the surface. These experiments show that the second magnet or electromagnet positioned above the reaction vessel is not necessary; thus simplifying the construction of the apparatus.

This approach could be extended to solutions containing any number of analytes by depositing the appropriate number of sensing coils on the substrate, applying appropriate capture layers and using suitably coated magnetic particles in the reaction vessel. A ceramic substrate with 5 sensing coils could be used.

Use of a Non-Pulsed External Magnetic Field To Accelerate the Reactions

In a further development, we have found that it is not necessary to pulse the magnetic field after the sample is added to the reaction vessel. A static field could be used. The sample procedure was the same as before, and 120 μl phosphate buffered saline was place into the vessel and recording of the resonant frequency of the sensing coil started. This gave the background drift of the resonant frequencies of the coils. After three minutes, 10 μl coated magnetic particles and 10 μl of the standard solution was added to the well and the electromagnet that pulled the paramagnetic particles onto the surface was switched on after a further 30 seconds (Figure 12). This time, a steady, non-pulsed, magnetic field was applied via the studs. Again, 60 seconds (t_1) after adding the particles, the slope of the recorded signal was determined over the next 100 seconds, ($t_2 - t_1$). The results were calculated in the same as before by plotting the difference in the slope of the recorded signal before the addition of paramagnetic particles (line AB) and that recorded over the ($t_2 - t_1$) period (line CD). This measurement method produced very similar results to those obtained when a pulsed magnetic field was used (Figure 13). These results were unexpected as it was assumed that it was necessary to switch off the magnetic field for some periods to allow unbound magnetic particles to move away from the reaction surface so that they did not interfere with the readings of the sensing coil. A possible explanation for this is that the magnetic particles bound to the reaction surface as a result of the assay interact much more strongly with the electromagnetic field created by the flat, spiral, sensing coil in the reaction vessel than the magnetic particles that are unbound. This is a result of two factors:

a. The electromagnetic field produced by the flat spiral coil.

b. The difference between the magnetic characteristics of the bound and unbound magnetic particles.

A simple calculation shows that the sensing coils produce intense, localised, electromagnetic fields that do not penetrate much beyond the reaction surfaces on the substrate. An approximate value for the variation in the magnetic field H_z along the central axis, z, of the coil can be determined by applying Biot-Savart's Law to the coil. For a circular coil with a single turn of radius a, the magnetic field produced by the coil carrying a certain current decreases with z by:

$$H_z = ka^2/(z^2 + a^2)^{1.5}$$

where k is a constant that depends on the current passing through the coil and the magnetic permeability of the medium surrounding the coil. A spiral, coil consisting of m turns may be approximated to m concentric coils with radii, a_1, a_2, a_3...and a_m. As the same current passes through each turn of the coil, the total field produced by the spiral coil is:

$$H_z = k \sum a_m^2/(z^2 + a_m^2)^{1.5}$$

where the summation is from $m = 1$ to m. This equation shows that the magnetic field strength falls rapidly with distance along the central axis of the coil. Applying the above equation to the coils used in these investigations shows that the magnetic field decreases rapidly with distance from the centre of the coil (Figure 14). If the substrate is 1mm thick, then the intensity of the magnetic field for particles bound to the reaction surface is about 10 greater than for particles that are 1mm away from the surface. The calculation shows that the magnetic field created by the sensing coil, and hence the inductance of

the coil, will be more strongly influenced by the particles on the reaction surface than the ones further away in the buffer solution. This model is a simplification as it deals only with the magnetic field and neglects such things as the permittivity of the substrate and the buffer solution. It also neglects the electric field associated with the rapidly oscillating magnetic field which will be affected by the high dielectric constant and high conductivity of the of the buffer solution: if these are taken into account then it is likely that the electromagnetic field created by the sensing coil falls even more rapidly than shown in Figure 14.

The particles used in these experiments have magnetic domains. The studs on the electromagnet or permanent magnet are positioned below the sensing coil and produce an intense, localised, magnetic field with a rapidly increasing flux density that induces magnetic moments into the particles and attracts them towards the reaction surface (Figure 15). Magnetic particles with attached analyte molecules bind quantitatively to the capture molecules on the reaction surface and the particles become immobilised. The magnetic domains in the immobilised particles will attempt to align along the direction of the magnetic field created by the stud. Collectively then, the immobilised particles have a large magnetic moment on the reaction surface which produces a significant perturbation in the intense, high-frequency magnetic field produced by the sensing coil, resulting in an increase in the inductance of the coil and a decrease in the resonant frequency. The unbound magnetic particles, however, are free to move and are subject to Brownian motion. In an extreme case, an unbound particle with its magnetic moment aligned along the steady field produced by the stud could be rotated by Brownian motion through 180° so that the moment is now in the wrong direction; the domain could flip its orientation by a process called Néel relaxation, but this is a relatively slow process compared with Brownian

motion; so it is likely that the particle will have to wait until the Brownian motion returns the magnetic moment to alignment. Thus the magnetic moments of the unbound magnetic particles are much more randomly orientated than the bound particles and so have a smaller effect on the magnetic field produced by the sensing coil. Unbound particles further away from the reaction surface will also be affected by Brownian motion and, as the field produced by the studs is weaker here than the surface, the magnetic moments of these particles will be more strongly influenced by the Brownian motion. The high-frequency field produced by the sensing coil is also weaker further away from the reaction surface and so these unbound particles produce a negligible response in the sensing coil. In this way, the sensing system is able to distinguish between bound and unbound particles.

CLAIMS

1. A magnetic particle detector system comprising a plurality of reaction surfaces; a corresponding plurality of electrical coils, each located adjacent a respective reaction surface; and a measuring system for measuring the effect of magnetic particles bound to a reaction surface on the inductance of the associated electrical coil.

2. A system according to claim 1, wherein the measuring system comprises a plurality of capacitors, each capacitor connected to a respective electrical coil to define a resonant circuit; a plurality of oscillators, each oscillator being coupled to a respective resonant circuit; and a monitoring system for determining the resonant frequencies of the resonant circuits.

3. A system according to claim 2, wherein the or each oscillator comprises one of a Colpitts, Hartley, or Armstrong oscillator.

4. A system according to claim 1, wherein the measuring system comprises a plurality of capacitors, each capacitor being connected to a respective electrical coil to define a resonant circuit; and a plurality of phase locked loops (PLLs), each PLL being connected to a respective resonant circuit to cause the circuit to resonate; and a monitoring system for monitoring the resonant frequency of, or a change in resonant frequency of, each resonant circuit.

5. A system according to claim 1, wherein the measuring system comprises a plurality of capacitors, each capacitor being connected to a respective electrical coil to define a resonant circuit; a phase locked loop (PLL); a switch for selectively connecting the PLL to each resonant circuit to cause the resonant circuit to resonate; a controller for actuating the switch; and a monitoring system for monitoring the resonant frequency of, or a change in resonant frequency of, each resonant circuit.

6. A system according to claim 5, wherein the capacitance of each capacitor can be varied by the controller.

7. A system according to claim 1, wherein the measuring system comprises a capacitor; a switch for selectively connecting the capacitor to each coil to form a resonant circuit; a phase locked loop (PLL) connectable to the resonant circuits via the switch; a controller for actuating the switch; and a monitoring system for monitoring the resonant frequency of, or a change in resonant frequency of, each resonant circuit.

8. A system according to claim 1, wherein the measuring system comprises a plurality of variable capacitors, each capacitor being connected to a respective electrical coil to define a respective test resonant circuit; a plurality of phase locked loops (PLL), each phase locked loop being connected to a respective test resonant circuit to cause the circuit to resonate, and to a respective mixer circuit; a reference resonant circuit and a reference PLL for causing the reference resonant circuit to resonate; each mixer circuit mixing, in use, the resonant frequency of the reference resonant circuit with the resonant frequency of the corresponding test resonant circuit to produce a beat frequency; and a switch to which the beat frequencies are supplied so as to be selectively connected to the measuring system; the measuring system including a controller for adjusting the variable capacitors and the switch.

9. A system according to claim 1, wherein the measuring system comprises a plurality of variable capacitors, each capacitor being connected to a respective electrical coil to define a resonant circuit; an oscillator common to all the resonant circuits; a plurality of phase detectors for detecting the phase differences between the signal from the oscillator and corresponding resonant circuit frequencies and generating a corresponding output signal; a switch to which the output signals are fed and being actuable selectively to supply the output signals to a monitoring system; and a controller for controlling the switch and adjusting the capacitors in response to the output signals from the phase detectors.

10. A system according to any of the preceding claims, wherein the coils comprise planar spirals.

11. A system according to any of the preceding claims, wherein each coil is wire wound.

12. A system according to any of the preceding claims, wherein the plurality of reaction surfaces are provided in a reaction vessel or on a test strip.

13. A binding assay device for detecting magnetic particles bound to a number of reaction sites of a system according to any of the preceding claims, the output of the measuring system being indicative of the quantity of magnetic particles bound to the reaction sites.

14. A device according to claim 13, wherein one of the coils is adjacent a reaction site to which magnetic particles cannot be bound so as to act as a reference site.

15. A device according to claim 13 or claim 14, further comprising a processor for computing the number of particles bound to a reaction site from the measured effect on the inductance of the coil.

16. A device according to any of claims 13 to 15, wherein one or more of the reaction sites includes a plurality of binding partners attached thereto.

17. A device according to any of claims 13 to 16, further comprising a magnetic field generator for generating a magnetic field in the vicinity of the reaction sites to attract magnetic particles towards the reaction sites.

18. An immunoassay device according to any of claims 13 to 17.

19. A method of performing a binding assay using a device according to any of claims 13 to 18, the method comprising providing different binding partners at the reaction sites; supplying a sample to the reaction vessel or test strip; supplying coated magnetic particles which attach to the binding partners via respective target analytes in the sample whereby the magnetic particles are caused to attach to the reaction sites in the presence of the target analytes in the sample; and monitoring the effect of

magnetic particles bound to the reaction surfaces on the inductance of the coils to detect the quantity of magnetic particles attached to the reaction sites.

20. A method of performing a binding assay using a device according to any of claims 13 to 18, the method comprising providing different binding partners at the reaction sites; supplying a sample to the reaction vessel or test strip; supplying coated magnetic particles which attach to the binding partners whereby the magnetic particles are caused to attach to the reaction sites and whereby, in the presence of the target analytes in the sample, the magnetic particles are caused to detach from the reaction sites due to preferential binding for the target analytes; and monitoring the affect of magnetic particles bound to the reaction surfaces on the inductance of the coils to detect the quantity of magnetic particles attached to the reaction sites.

21. A method of performing a binding assay using a device according to any of claims 13 to 18, the method comprising providing different binding partners at the reaction sites; supplying a sample to the reaction vessel or test strip, the sample including at least one target analyte which is complementary to the binding partners at one of the reaction sites; supplying at least one competitor to which magnetic particles are attached, the or each competitor being complementary to the binding partners at one of the reaction sites, whereby the or each target analyte inhibits the binding of its respective competitor and attached magnetic particles to the respective reaction site; and monitoring the effect of magnetic particles bound to the reaction sites via the or each competitor on the inductance of the coils to detect the quantity of magnetic particles attached to the reaction sites.

22. A method of performing a binding assay using a device according to any of claims 13 to 18, the method comprising providing different competitors at the reaction sites; supplying a sample to the reaction vessel or test strip,

the sample including at least one target analyte; supplying coated magnetic particles which are provided with binding partners which are complementary to at least one target analyte and to its respective competitor, whereby the or each target analyte inhibits the binding of the magnetic particles to the respective reaction site via its respective competitor; and monitoring the effect of magnetic particles bound to the reaction sites via the or each competitor on the inductance of the coils to detect the quantity of magnetic particles attached to the reaction sites.

23. A method according to claim 22 wherein the coated magnetic particles are provided with binding partners which are complementary to more than one target analyte and their respective competitors.

24. A method according to any of claims 19 to 23, when dependent on claim 12, wherein the magnetic particles are supplied to the reaction vessel or test strip at the same time as the sample.

25. A method according to any of claims 19 to 24, when dependent on claim 12 and any of claims 6 to 9, wherein, prior to supplying the sample to the reaction vessel or test strip, the resonant frequency of each circuit is adjusted so that the detected phase difference is substantially zero.

26. A method according to any of claims 19 to 25, wherein the binding partners and target analytes are biological or chemical substances.

27. A method according to claim 26, wherein the binding partners and target analytes are chosen from antibody and corresponding antigen, hormone and hormone receptor, hormone and hormone binding protein, drug and drug receptor, enzyme and cofactor, transcription factor and DNA, subunits of a protein complex such as G-proteins, any signalling or transport protein and its control element, lectins and glycoproteins, lectins and carbohydrate moities, receptor protein and lipoprotein, lipid and

lipoprotein, DNA and DNA, DNA and RNA, RNA and RNA, PNA and PNA, PNA and DNA, PNA and RNA, cell membrane proteins and virus, cell membrane proteins and spores, cell membrane proteins and bacteria.

28. A method according to any of claims 19 to 27 wherein the sample comprises a biological or chemical substance.

29. A method according to claim 28, wherein the sample comprises one of whole blood, plasma, serum, urine, a water sample, for example from a river, reservoir or lake, sewage, farm run-off, soil and plant extract.

Fig.1.

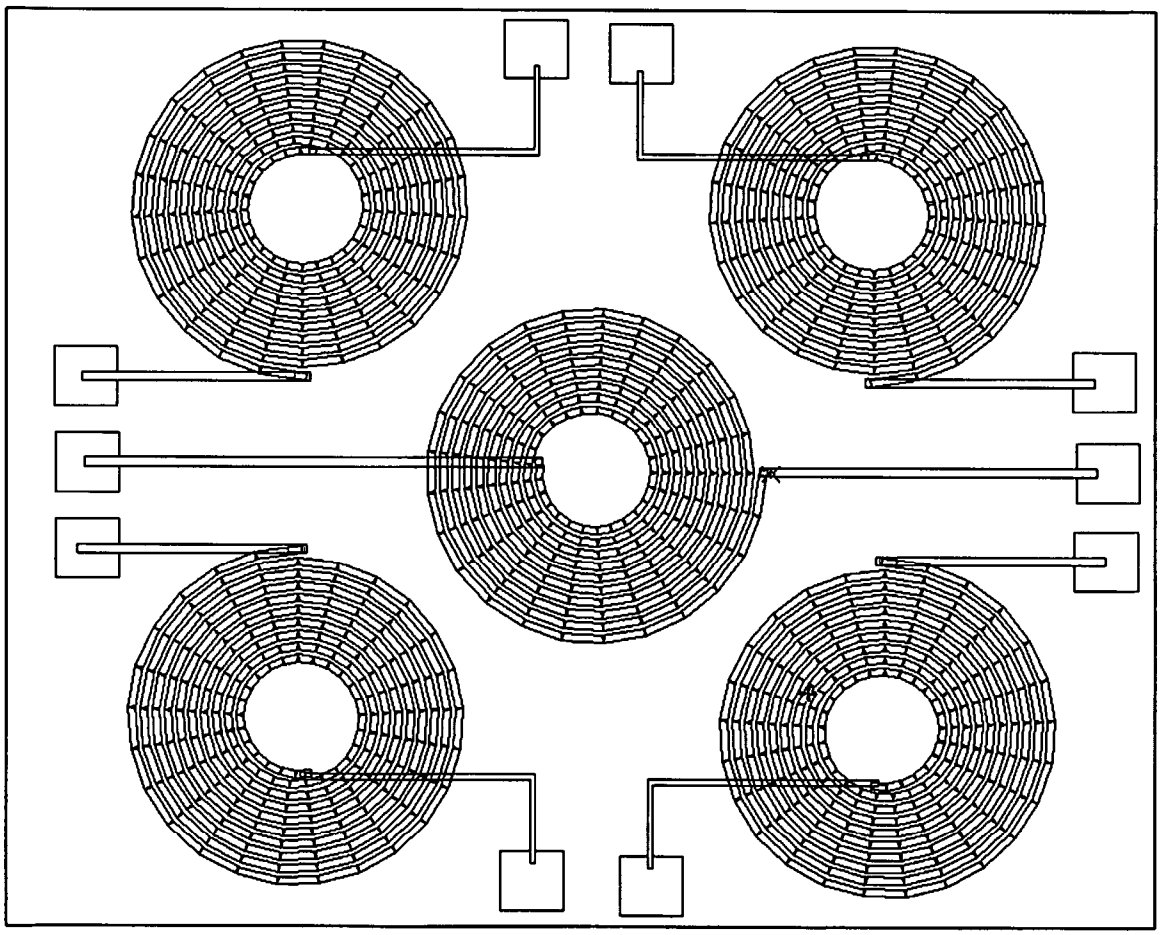


Fig.2(a).

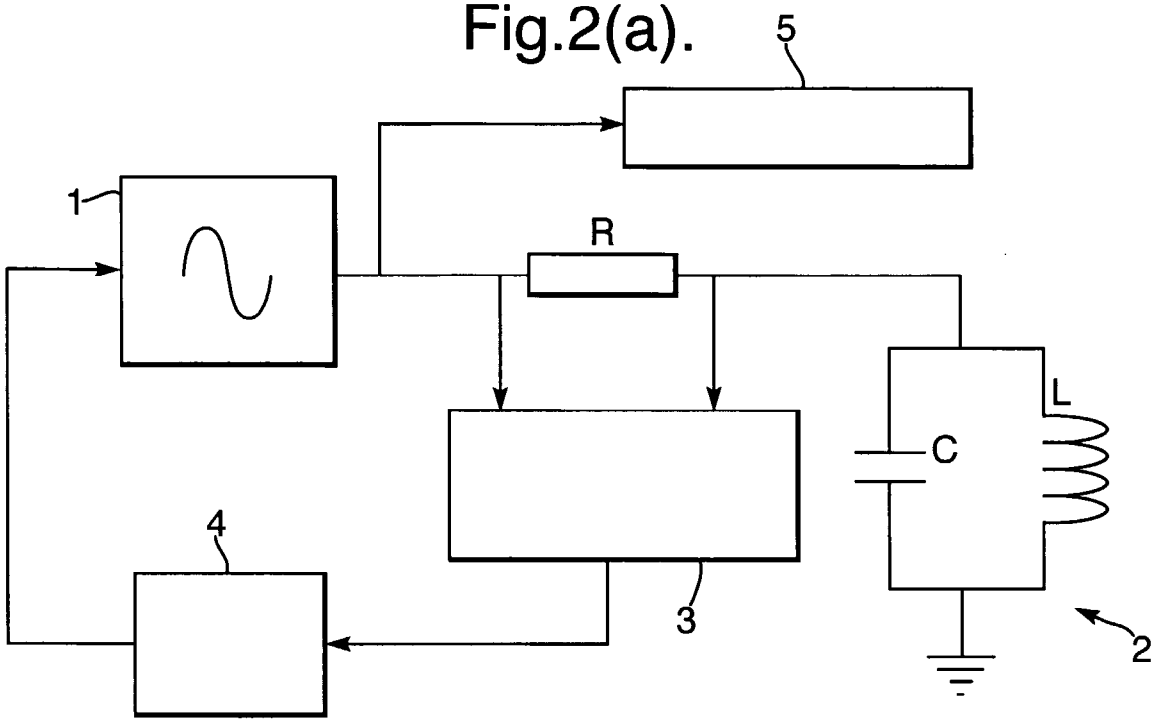


Fig.2(b).

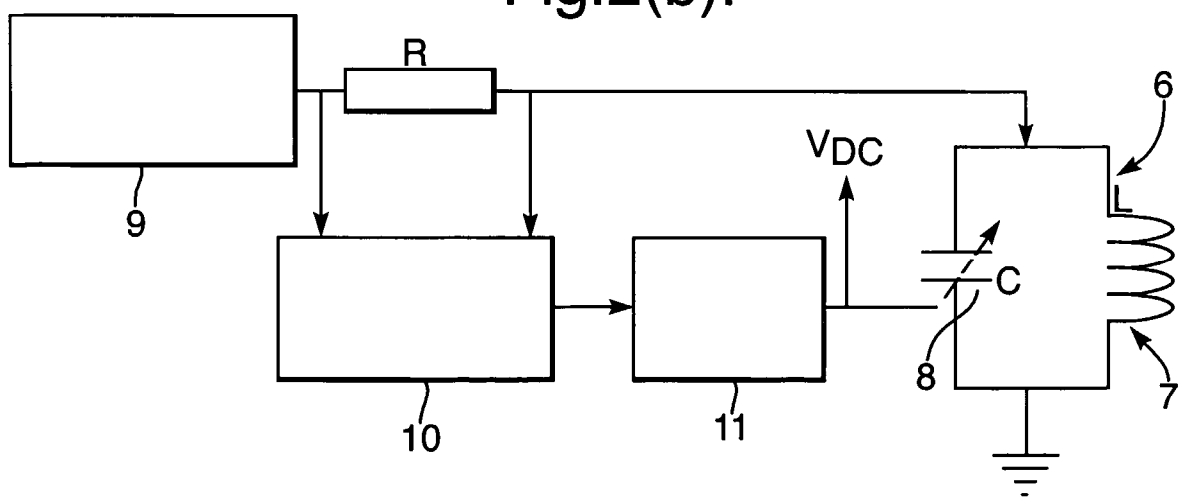


Fig.2(c).

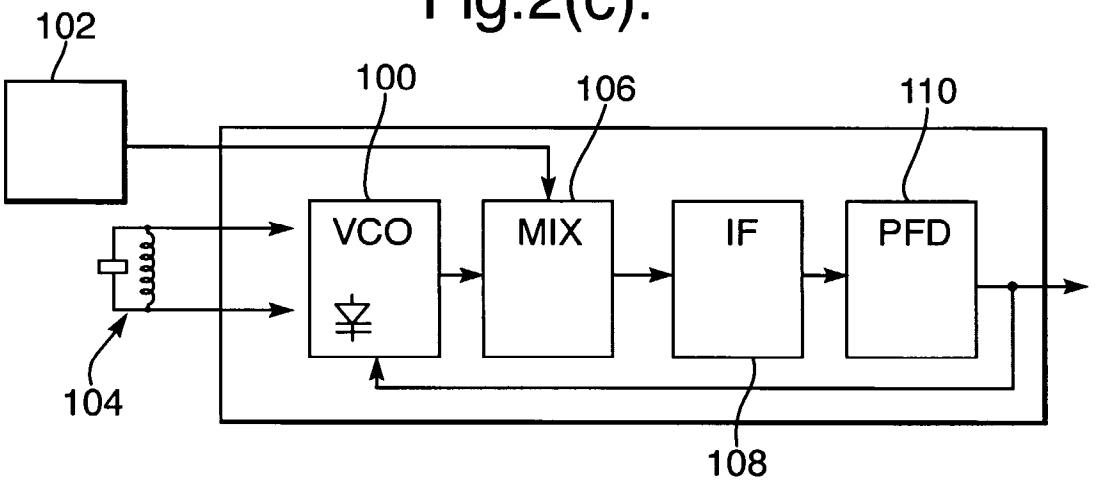


Fig.3.

$r^2 = 0.98$

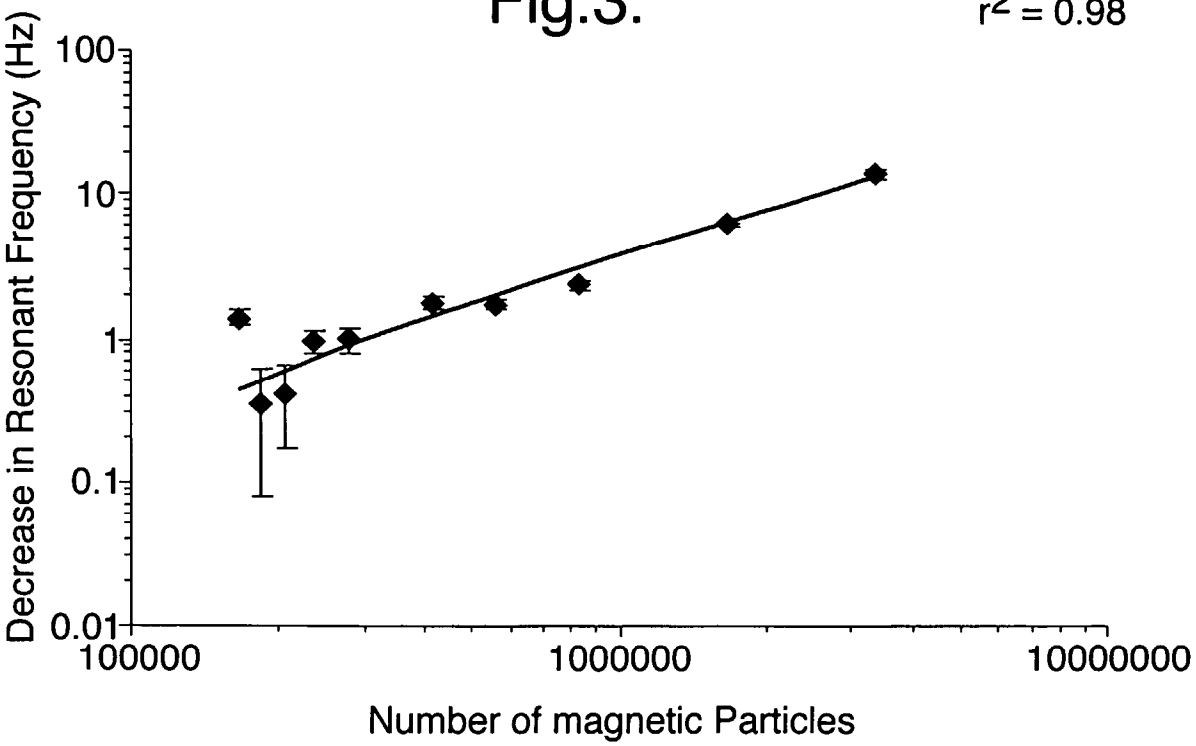


Fig.4.

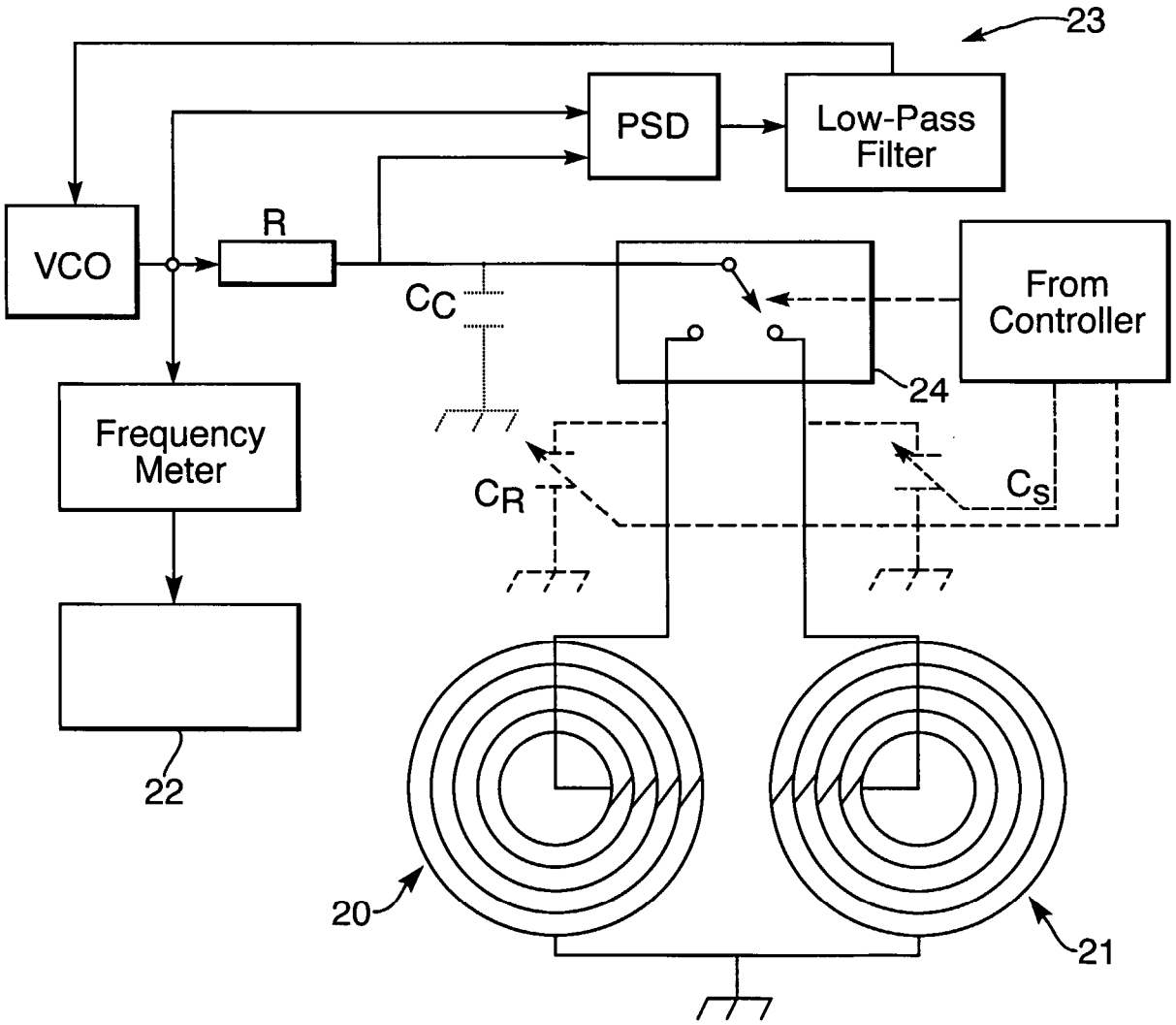

23
PSD
Low-Pass Filter
VCO
R
CC
From Controller
24
Frequency Meter
CR
CS
22
20
21

Fig.5.

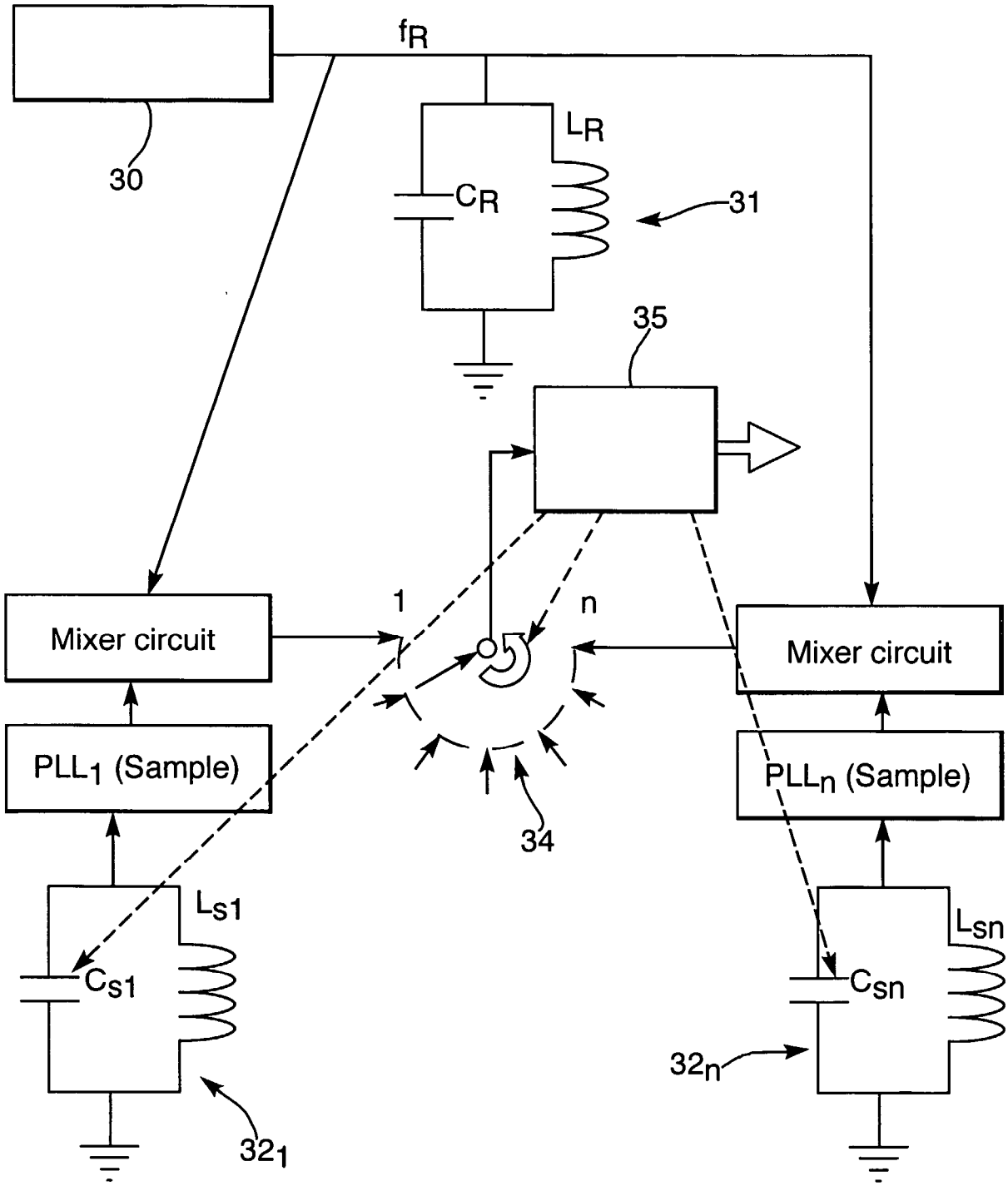

fR
30
LR
CR
31
35
1
n
Mixer circuit
Mixer circuit
PLL1 (Sample)
PLLn (Sample)
34
Ls1
Cs1
Lsn
Csn
32n
321

Fig.6.

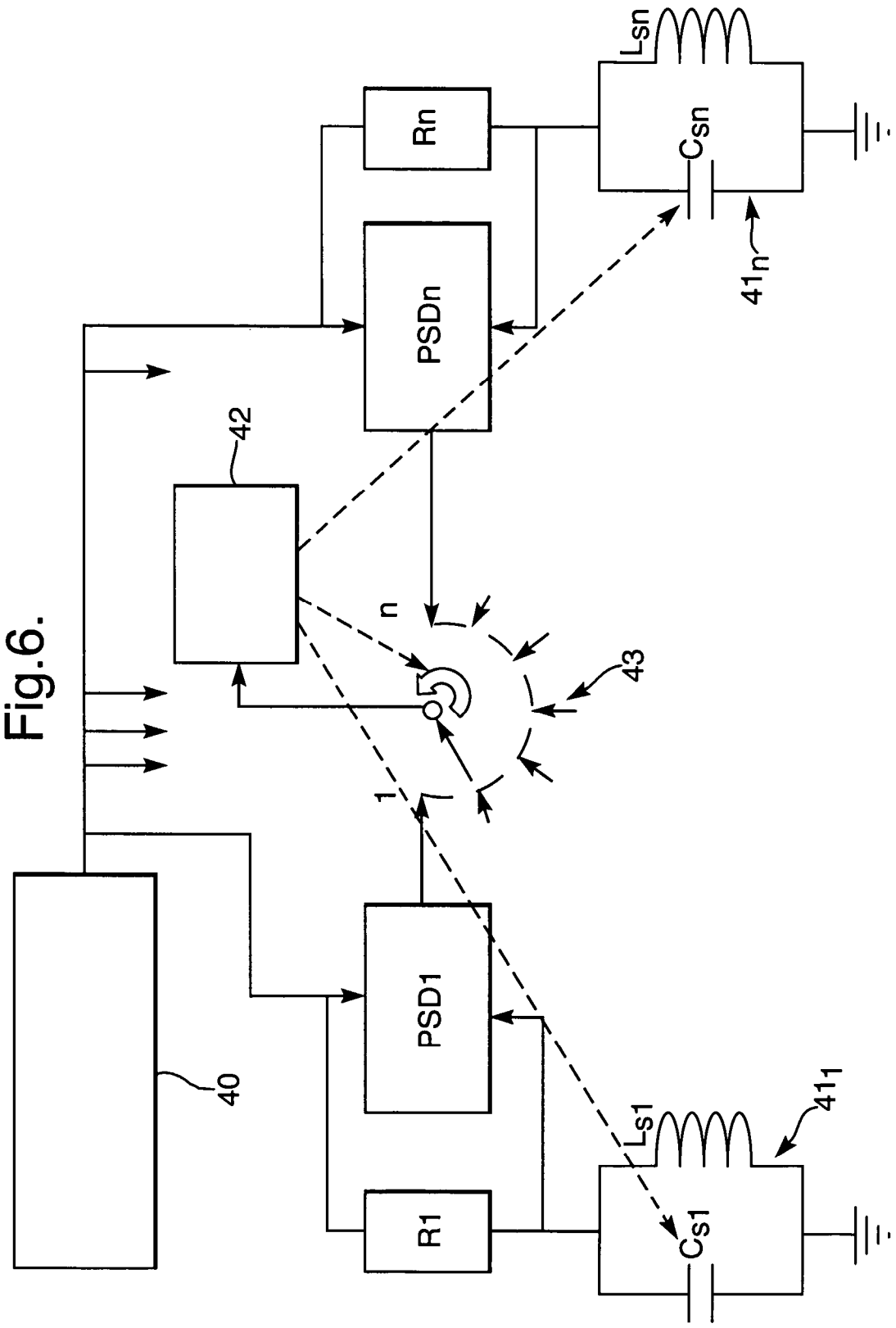


Fig.7.

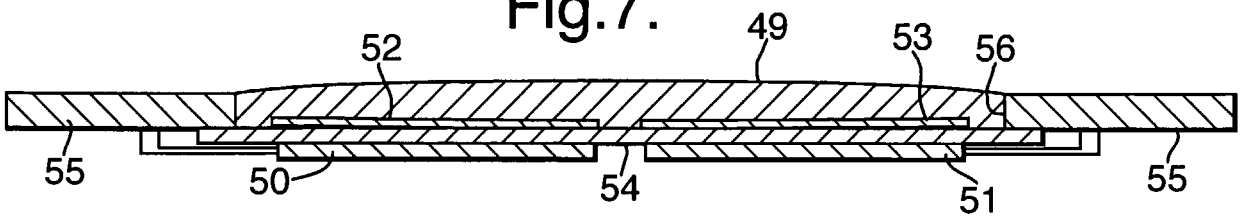


Fig.8.

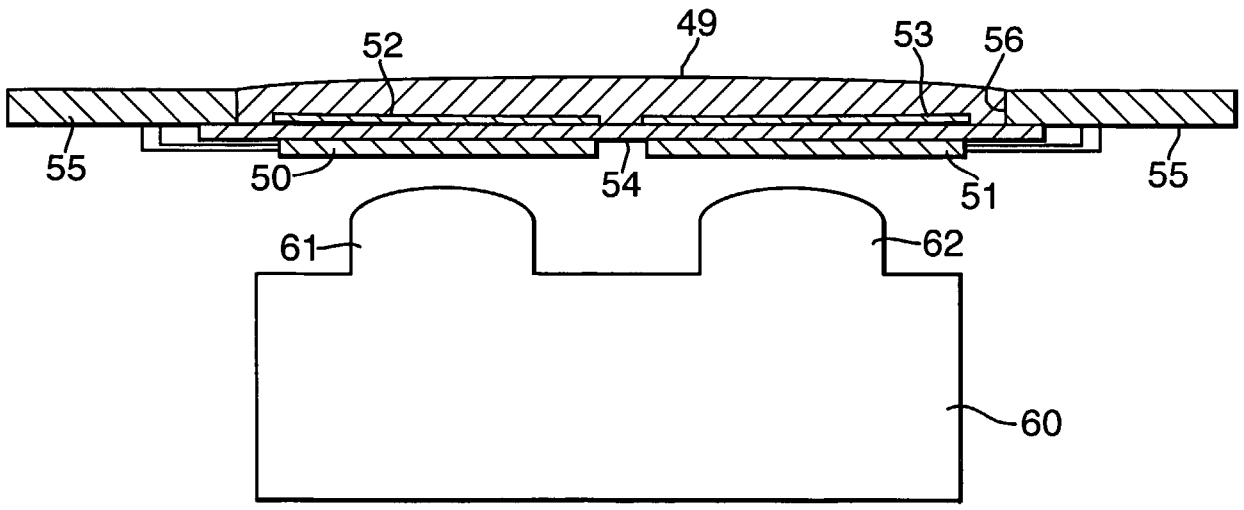


Fig.7a.

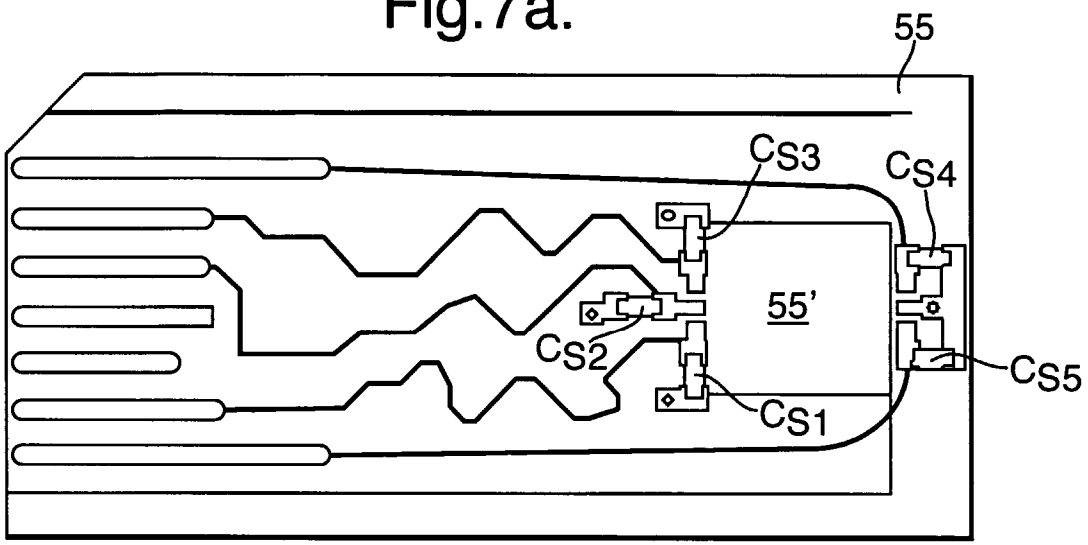

55
CS3
CS4
55'
CS2
CS5
CS1

Fig.10.

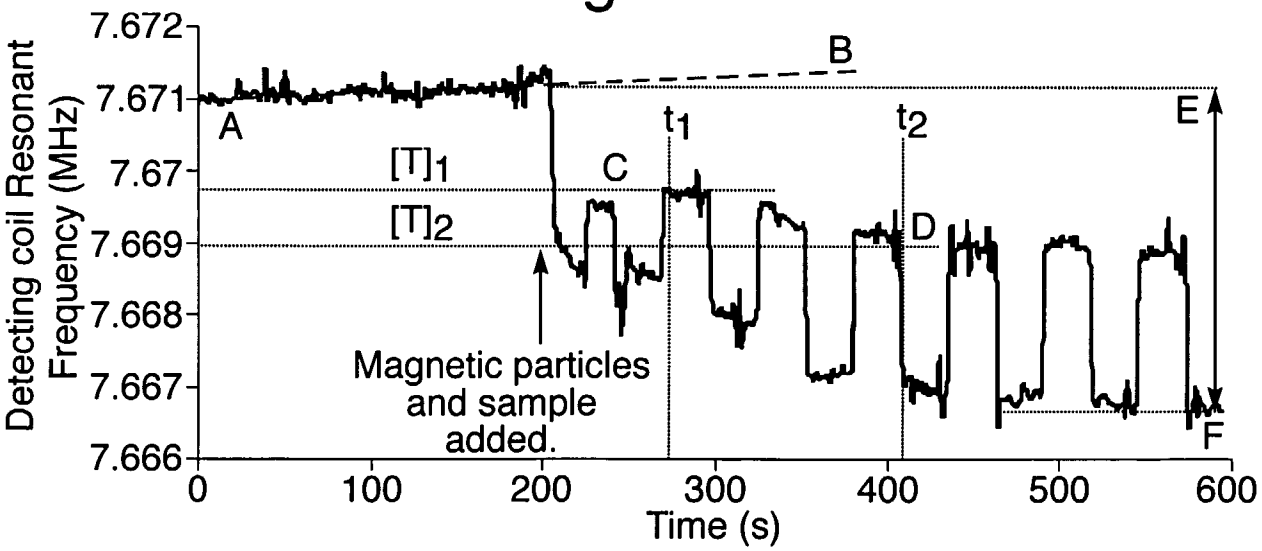

Detecting coil Resonant Frequency (MHz)
7.672
7.671
7.67
7.669
7.668
7.667
7.666
A
B
C
D
E
F
[T]1
[T]2
t1
t2
Magnetic particles and sample added.
0
100
200
300
400
500
600
Time (s)

Fig.9.

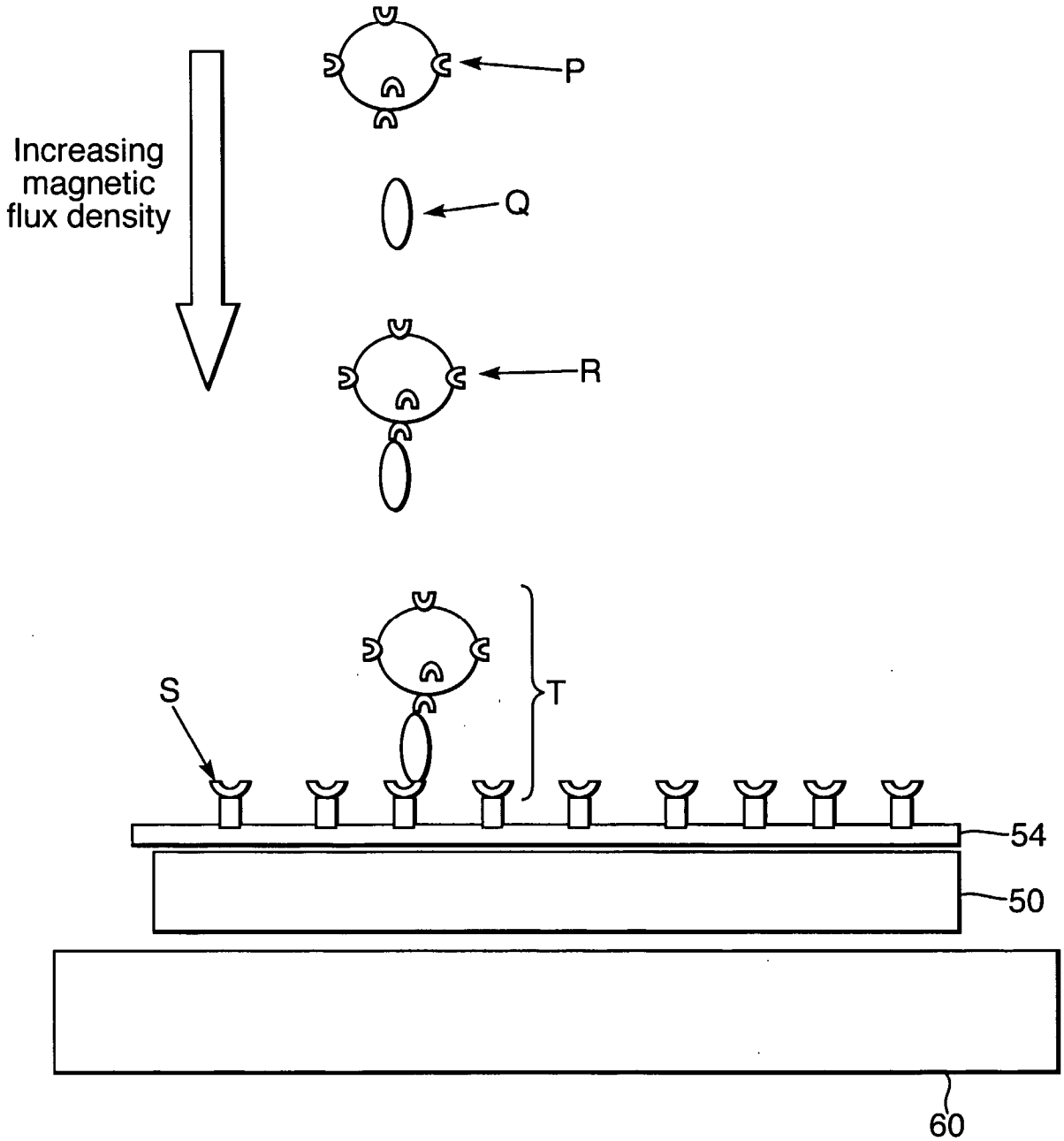


Fig.11.

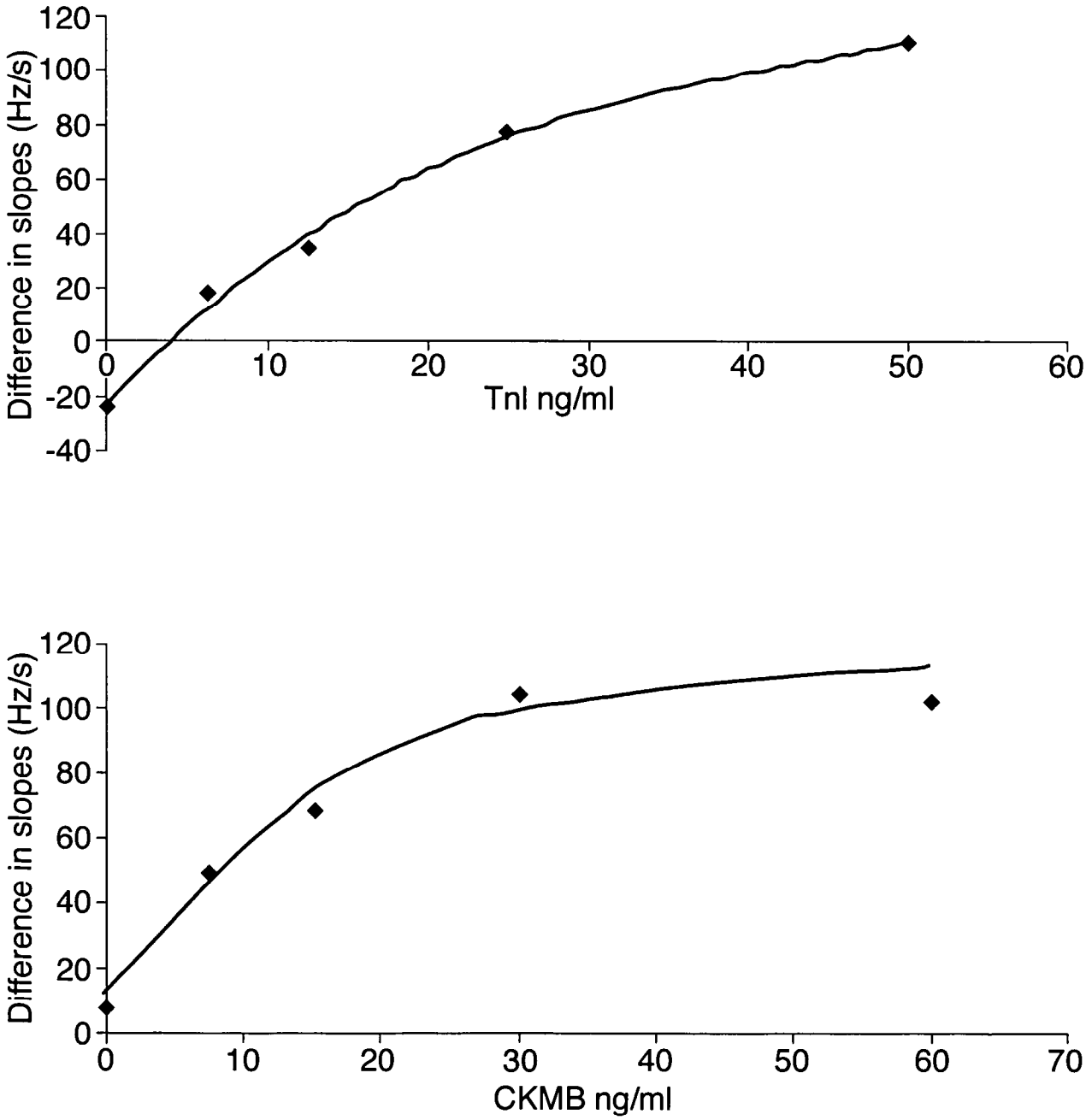

Difference in slopes (Hz/s)
120
100
80
60
40
20
0
-20
-40
0
10
20
30
40
50
60
TnI ng/ml
Difference in slopes (Hz/s)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
CKMB ng/ml

Fig.12.

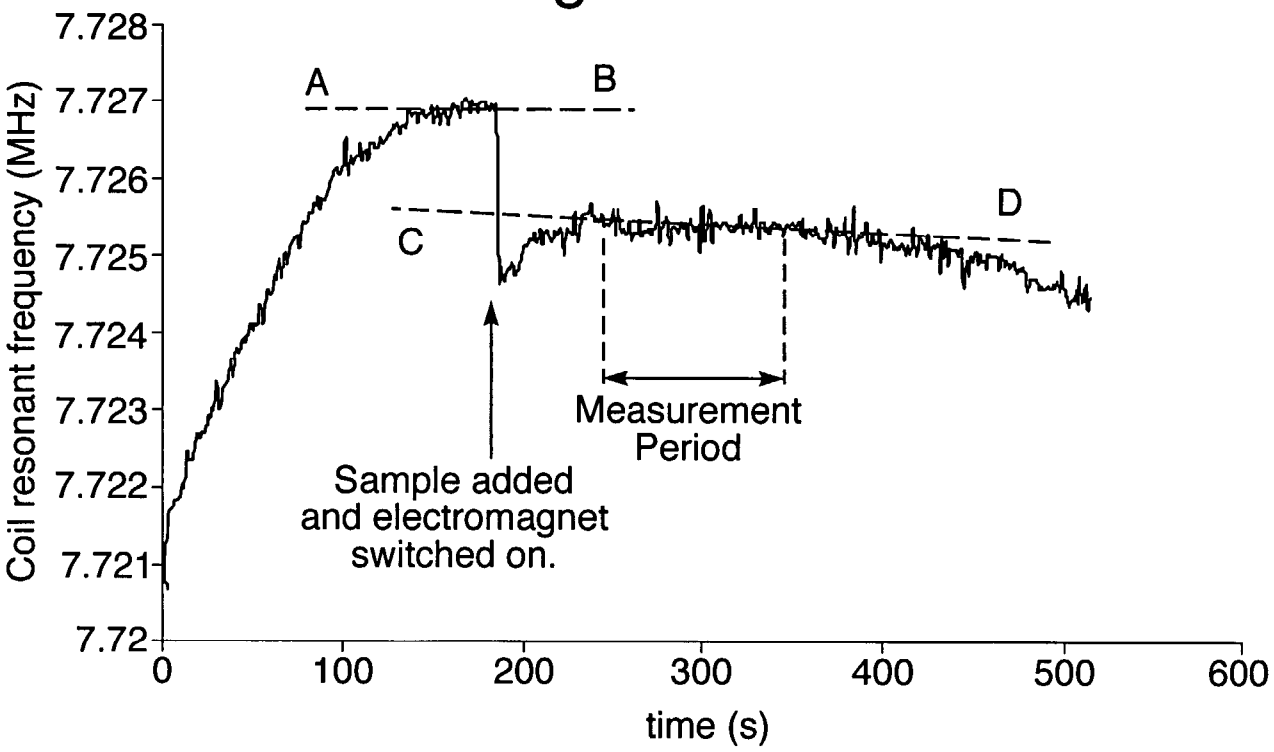

7.728
7.727
7.726
7.725
7.724
7.723
7.722
7.721
7.72
Coil resonant frequency (MHz)
A
B
C
D
Measurement Period
Sample added and electromagnet switched on.
0
100
200
300
400
500
600
time (s)

Fig.13.

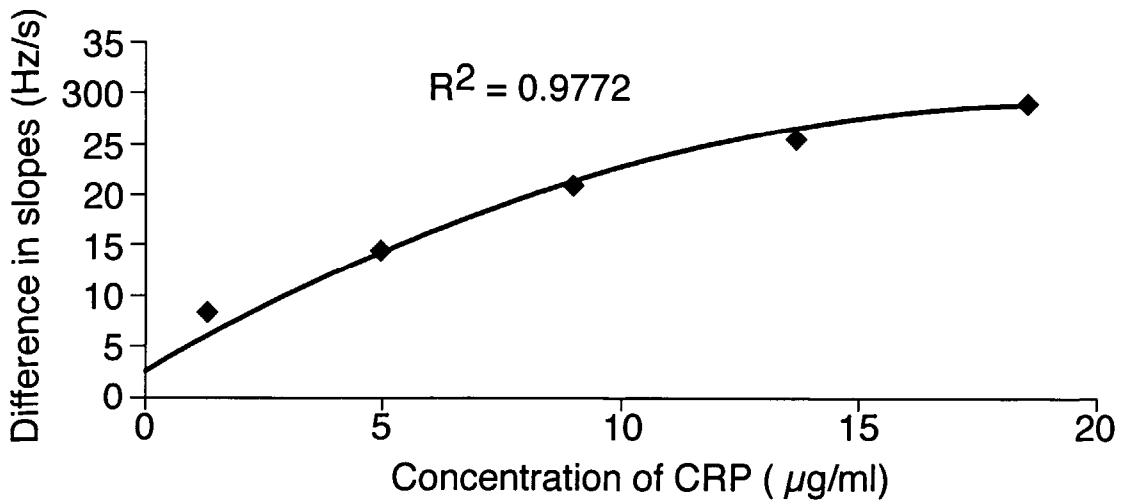

35
300
25
20
15
10
5
0
Difference in slopes (Hz/s)
R2 = 0.9772
0
5
10
15
20
Concentration of CRP (µg/ml)

Fig.14.

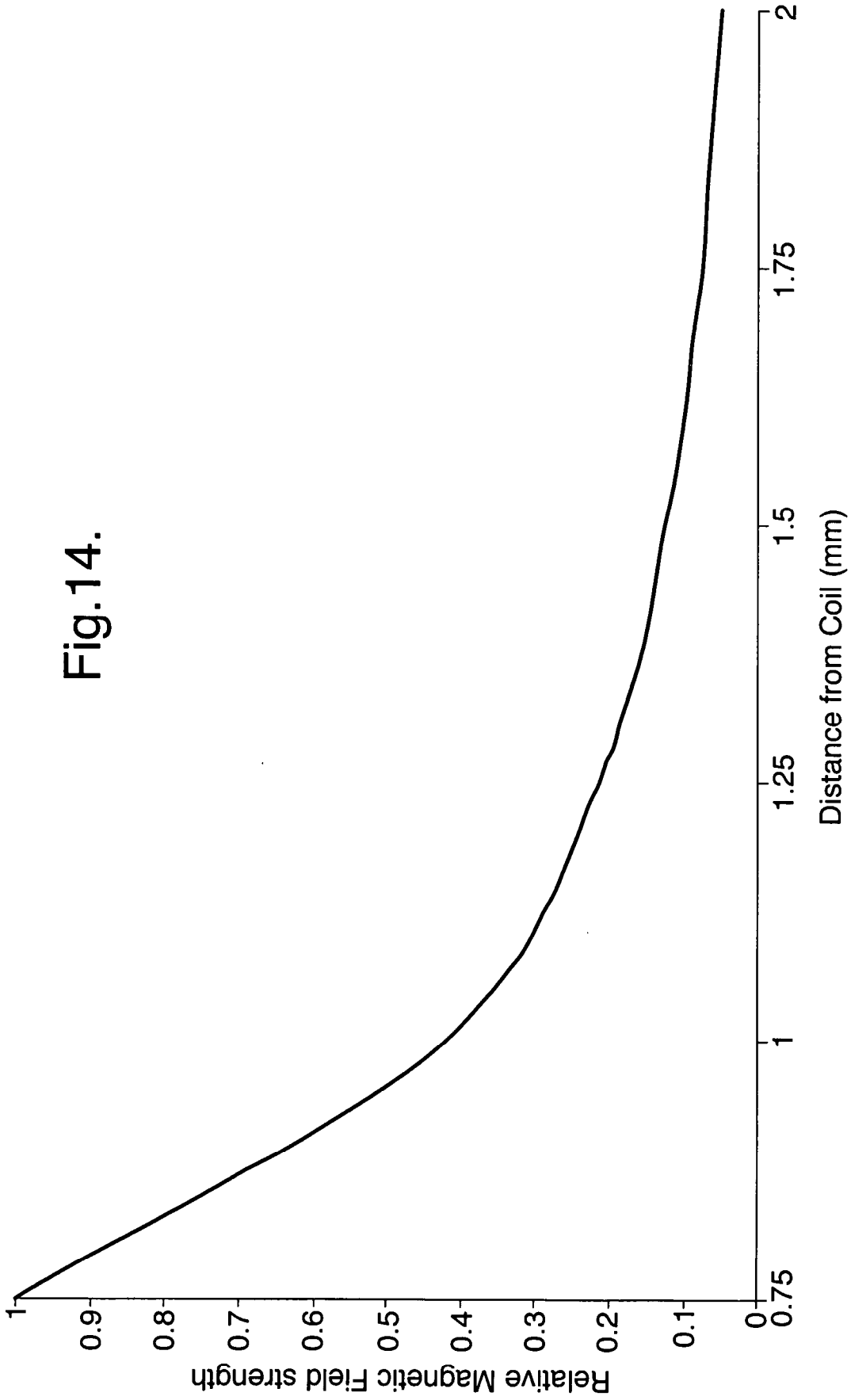
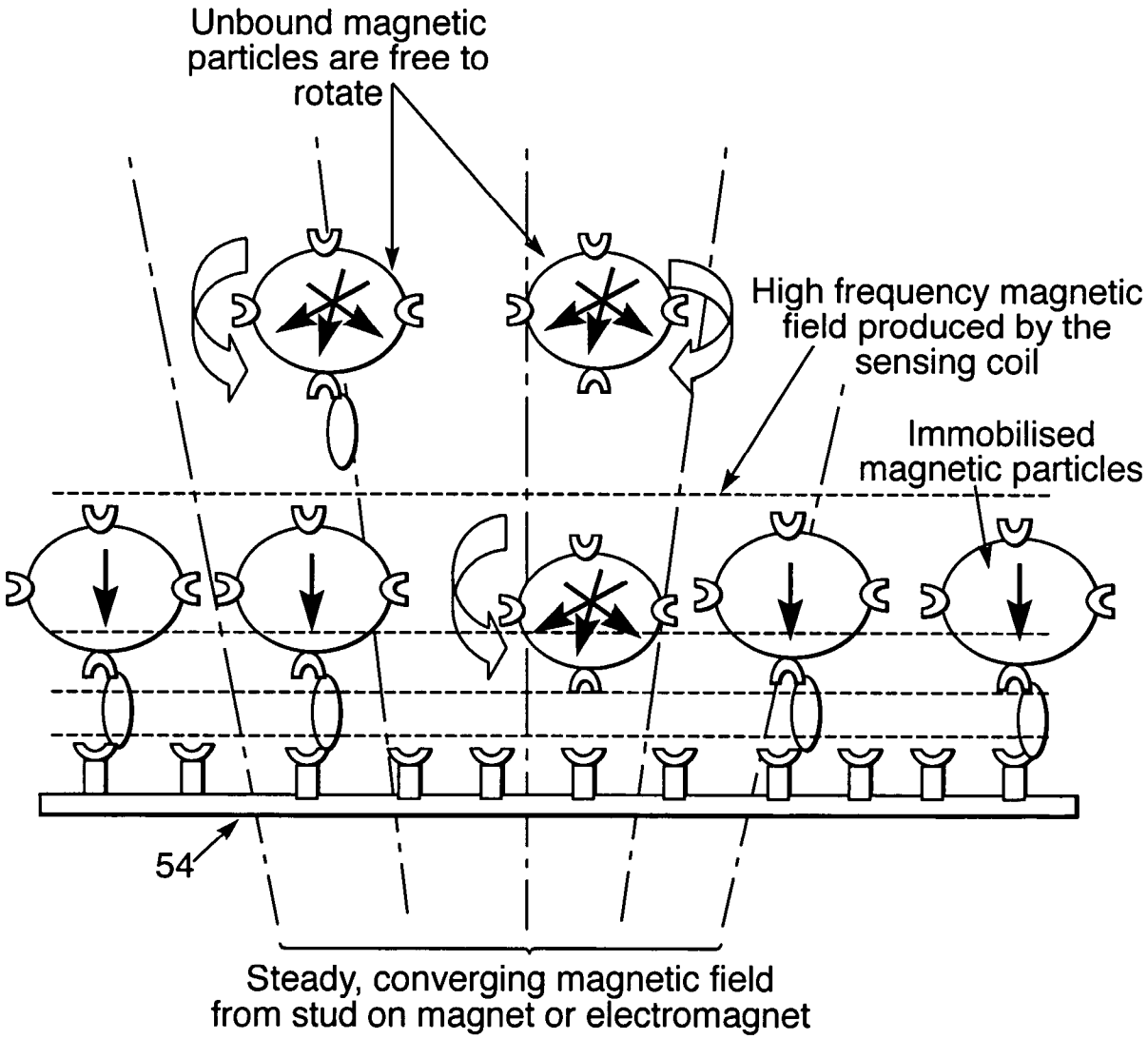

Relative Magnetic Field strength
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.75
1
1.25
1.5
1.75
2
Distance from Coil (mm)

Fig.15.


Unbound magnetic particles are free to rotate
High frequency magnetic field produced by the sensing coil
Immobilised magnetic particles
54
Steady, converging magnetic field from stud on magnet or electromagnet

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB2005/001936

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/543 G01N27/72 G01R33/12

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N G01R

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2003/012693 A1 (OTILLAR ROBERT ET AL) 16 January 2003 (2003-01-16) the whole document	1,10-13, 15-19, 24,26-29 2-9,14, 20-22

X	WO 02/31505 A (AVIVA BIOSCIENCES CORPORATION) 18 April 2002 (2002-04-18) the whole document	1,10-13, 15-19, 24,26-29

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2005	29/07/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Diez Schlereth, D

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INTERNATIONAL SEARCH REPORT

Interi al Application No
PCT/GB2005/001936

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LUXTON R ET AL: "USE OF EXTERNAL MAGNETIC FIELDS TO REDUCE REACTION TIMES IN AN IMMUNOASSAY USING MICROMETER-SIZED PARAMAGNETIC PARTICLES AS LABELS (MAGNETOIMMUNOASSAY)" ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. COLUMBUS, US, vol. 76, no. 6, 15 March 2004 (2004-03-15), pages 1715-1719, XP001196657 ISSN: 0003-2700 the whole document	2-9,14, 20-22
Y	RICHARDSON JULIE ET AL: "A novel measuring system for the determination of paramagnetic particle labels for use in magneto-immunoassays" BIOSENSORS AND BIOELECTRONICS, vol. 16, no. 9-12, December 2001 (2001-12), pages 1127-1132, XP002335571 ISSN: 0956-5663 the whole document	2-9,14
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INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/GB2005/001936

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