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(71) Applicant: **THE SECRETARY OF STATE FOR DEFENCE** [GB/GB]; DSTL, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).(72) Inventors: **WHITE, Peter John**; DSTL IP Group, G01-B Building 005, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). **WALKER, Maurice Daverell**; DSTL IP Group, G01-B Building 005, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). **RACHWAL, Phillip Anthony**; DSTL IP Group, G01-B Building 005, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).(74) Agent: **FARNSWORTH, Alastair Graham**; DIPR Formalities Section, Poplar 2#2214, MOD Abbey Wood (South), Bristol BS34 8JH (GB).

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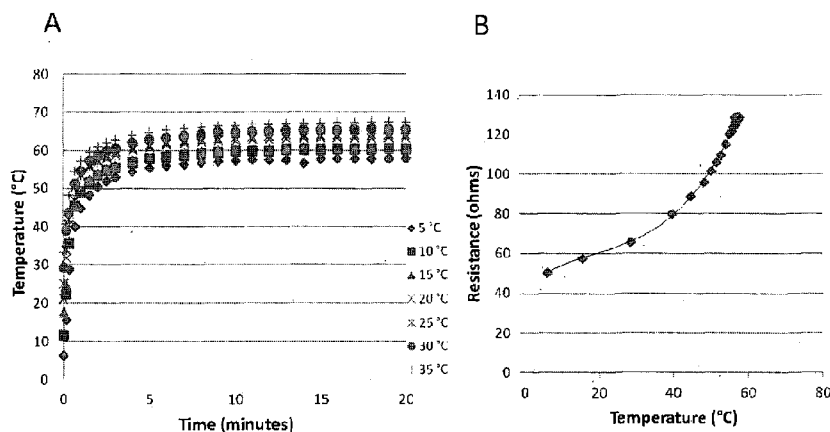
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(54) Title: METHOD AND DEVICE FOR HEATING A CHEMICAL REACTION

Figure 1



(57) **Abstract:** The present invention provides methods and devices for heating biological, chemical and/or enzymatic reactions, in particular isothermal nucleic acid amplification reactions. The devices may in particular be hand-held, fieldable, portable and/or disposable.

Method and Device for Heating a Chemical Reaction

The present invention is concerned with methods and devices for heating chemical, biological or enzymatic reactions at a predetermined temperature, especially isothermal nucleic acid amplification reactions, and especially hand-held, fieldable devices for detection of nucleic acids through isothermal nucleic acid amplification.

There is forever a need for improved methods and devices for identification of chemical and biological agents in the field, and for the devices to be lightweight, hand-held, preferably disposable and with minimal or no electrical power requirement. A number of devices exist for identification of chemical and biological agents using antibody based assays, such as using lateral flow technology, such as the device described in GB patent No. 2460956. Though antibody based assays have great utility in this area, the sensitivity of some antibody assays can be a limiting factor. It is recognised that specific detection of genetic loci could provide a solution to some of the sensitivity issues of antibody assays through use of nucleic acid amplification. It is however also recognised that nucleic acid amplification assays require a heat source, for either thermocycling, or even for isothermal amplification, and consequently an electrical power source, which has made it a lot more difficult to devise a fieldable device, especially one which is hand-held and disposable.

Non instrumented nucleic acid amplification (NINA) is an aspiration for molecular diagnostics in low resource settings, for example home health care, developing-country healthcare and emergency situations, such as to detect a deliberate release of a biowarfare pathogen, such as if DNA amplification chemistry could be combined with simple hardware and detection chemistry in a low cost disposable device, especially for use by a non-specialist operator in a wide range of low resource settings.

Isothermal nucleic acid amplification reactions eliminate the need for closely controlled thermocycling associated with traditional PCR technology. The amplification reaction proceeds,

depending on the biochemistry of the polymerase, at an optimal temperature ranging from 35°C to 70°C. Controlling such reactions is therefore less complex than those that require thermocycling where the specificity of the reaction is driven by the precise thermodynamics of association, or hybridisation, between specific oligonucleotide primers and a melted target DNA sequence. The reduced need for heating and cooling in isothermal reactions has made them good candidates for exploitation in the development of genetic testing devices that can be used in resource limited environments, such as in the field. However, isothermal reactions still need some thermal control to maintain a constant optimal temperature, that supports amplification, appropriate for the polymerase used in the amplification reaction. In electrically powered laboratory instruments this can be achieved using thermal control devices e.g. thermocouples and temperature controllers that can drive heating and cooling using on/off, proportional or PID control. In such a system a constant reaction temperature is maintained regardless of the ambient temperature. The situation is however completely different for fieldable devices which should preferably have no or minimal requirement for use of an electrical power source.

Fieldable disposable devices could use phase change materials, such as those incorporating or undergoing exothermic chemical reactions, like the sodium acetate used in COTS hand warmers, to support the isothermal reaction. However, whilst this type of substance can deliver a stable thermal output, it is difficult to control when the ambient temperature is unknown or variable, and especially at ambient temperatures below 10°C.

One such method using exothermic chemical reactions coupled with a phase change material is that by Singleton J. et al (Proc SPIE, March 09, 2013, 8615: 86150R; PLOS ONE, November 26, 2014, 9(11), e113693, doi:10.1371) which uses LAMP (loop mediated isothermal amplification) DNA amplification. Here a heating chamber has been designed (having a sample tube holder in a vacuum flask) such that a heat pouch containing a magnesium/iron alloy is inserted into the chamber and then wetted with a saline solution. This produces a chemical reaction within a super corroding

galvanic cell that generates a large amount of heat. Thermal regulation in this device is achieved by coupling the heat produced to a phase change material e.g. palmitic acid. The reaction chamber takes approximately 12 minutes to achieve a target temperature of 61.5°C after which reaction tubes containing the DNA amplification assays are added. The device is able to maintain the optimal reaction temperature at ambient temperatures in the range of 16 to 30°C.

Disadvantages of this system include the requirement for a multicomponent heating system comprising heat pouch, saline solution and phase change material that require several manipulations for final assembly and operation, and the fact that the exothermic chemical reaction produces magnesium hydroxide, hydrogen gas and steam, with the gases needing to be vented from the device to avoid over pressurisation. The release of hydrogen gas also poses a potential explosive hazard in a confined space. In addition, the reaction chamber only reaches target temperature after approximately 12 minutes, this relatively long lag time needing to be added to the total time needed to complete the amplification reaction, and the device is unable to maintain the optimal temperature at ambient temperatures below 16°C, or above 30°C.

Another exothermic chemical heating system reported by Lillis L et al (PLOS ONE, 29 September, 2014, 9(9), e108189, doi:10.1371) uses the RPA (recombinase polymerase amplification) isothermal DNA amplification method. RPA reactions typically work at a lower temperature (25 to 43°C) than LAMP reactions and do not require a stringent incubation temperature. In this system an exothermic reaction created by the crystallisation of liquid sodium acetate mixtures was used. However, at temperatures below 15°C detection was shown to be sporadic, especially during 20 minute incubation periods.

A further disadvantage of both the systems described is that they are large in size and would be difficult to develop into integrated systems that include sampling and specific detection. The first system uses a Thermos cup with an approximate volume of 314 cm³. The RPA system used 22 cm³ of

a sodium acetate solution in a small container that held a single reaction, and this volume did not include the insulation that was necessary for the system to function well.

Consequently, there remains a need to develop a truly cost effective, disposable, fieldable, nucleic acid detection device that uses isothermal amplification chemistry, and especially a device that can reproducibly function at a broad range of ambient temperatures, such as for example from below 0°C, to in excess of 40°C, but at least to be able to function reproducibly below 15°C and above 30°C.

As used herein, disposable generally refers to a single use device, i.e. a device which is used once and then disposed of (i.e. it is not reused), but can potentially also refer to a device which may be used more than once, but is still intended to be disposable.

The present invention thus generally aims to provide methods and devices for detecting nucleic acids through isothermal nucleic acid amplification which are suitable for detecting nucleic acids in the field, and which are preferably suitable to function at ambient temperatures below 15°C and above 30°C.

Accordingly, in a first aspect, the present invention provides a method for heating a chemical, biological, and/or enzymatic reaction at a pre-determined temperature comprising use of a positive temperature coefficient (PTC) heating element to control and maintain the temperature at the pre-determined temperature.

Chemical, biological and enzymatic reactions are processes that lead to the transformation or modification of a substance, such as the transformation of one chemical to another (a chemical reaction), or the transformation of a biological molecule (a biological reaction), which could be through the use of an enzyme (an enzymatic reaction). Clearly there is much overlap between chemical, biological, and enzymatic reactions, which are evidently all chemical reactions, but performed on different types of substances, or carried out by different agents (such as enzymes).

The method can however also be used for general heating in the fields of chemistry and biology, for example heating of a microbial or mammalian culture.

The Applicant has identified a simple, cheap, and low power means of maintaining a reaction at a given temperature without the need for associated thermostatic control elements. This novel use of PTC materials overcomes the significant problems of achieving thermostatic control of chemical, biological, or enzymatic reactions such as isothermal nucleic acid amplification reactions, and especially in field portable (fieldable) or hand held devices. Use of a positive temperature coefficient heating element is particularly capable of controlling the temperature of a reaction in environments that may have ambient temperatures far from the reaction optimum, such as below 0°C and in excess of 40°C, but optionally the predetermined temperature can be maintained at at least between 5°C and 35°C.

Thus in one advantageous embodiment of the method of the first aspect the pre-determined temperature is capable of being maintained in ambient temperatures of between 5°C and 35°C, and in another advantageous embodiment the pre-determined temperature is capable of being maintained in ambient temperatures below 5°C, and possibly below 0°C, for example 0°C, -5°C and -10°C, and optionally above 35°C, and possibly above 40°C, for example 40°C, and 45°C.

Positive Temperature Coefficient (PTC) heating elements (often termed PTC thermistors because they are essentially resistors with a positive temperature coefficient, which means that resistance increases with increasing temperature) are generally small ceramic chips with self-limiting temperature characteristics. PTC chips have fast heating response times and plateau once the pre-determined/pre-defined reference temperature is reached. Above the reference temperature, the semiconducting and ferro-electrical properties of the ceramic are utilized to produce a rise in resistance of several orders of magnitude, and thereby creating its self-limiting properties. The rise in resistance is experienced within a fairly small temperature window of a few degrees centigrade. This attribute of the PTC results in a heating element that self-regulates to a pre-set temperature

and automatically varies its wattage in order to maintain that pre-set temperature. In a given application, a greater degree of thermal dissipation (cooling) will result in a lower resistance being maintained by the element. This effect increases the power output of the heater during operation in colder temperatures. Inversely, as ambient temperature increases and less heat is dissipated, the resistance of the element will increase culminating in a near-zero current draw at its designated temperature.

The dynamic resistance and output of PTC heaters makes them an excellent choice for providing controlled electrical heating, especially in devices for use in the field, as such devices can be driven by small power supplies, for example alkaline or Lithium ion batteries, and are ideal for integrating into a simple, disposable device, as the size and weight of the heater and the batteries are small, and the cost low. The ceramic materials can be tailored to match the temperatures required in this reaction of interest; however the large scale industrial base for this technology offers bespoke solutions. This use of PTC heaters in particular overcomes the significant problems of achieving thermostatic control of isothermal nucleic acid amplification reactions in hand held frontline devices.

Accordingly, in one embodiment, the reaction is an isothermal nucleic acid amplification reaction.

An isothermal nucleic acid amplification reaction utilises a nucleic acid polymerase enzyme which is optimal at a specific pre-determined temperature. The PTC heating element should therefore be designed to provide a pre-determined temperature at or close to the optimal temperature of the polymerase enzyme.

Identification of a means of heating an isothermal nucleic acid amplification reaction which is of relatively low cost, with a low power requirement, and the elements are also lightweight and small, provides the ideal means for heating in a hand-held device, which can be used in the field, and which further can potentially be disposable.

Accordingly, in a second aspect, the present invention provides a device for detection of nucleic acids via isothermal nucleic acid amplification comprising a positive temperature coefficient heating element.

Embodiments of the device may be hand-held, fieldable, portable and/or disposable.

The device may comprise a PTC heating element with metal contact for connecting to a small battery power source such as an alkaline or lithium battery, similar to batteries used in watches. The PTC heating element may surround the base of a plastic container in which the amplification reaction is to occur.

The amplification vessel itself (i.e. the tube or well in which the reaction is performed) could be manufactured from PTC, and thus the vessel and the PTC heating element could be one and the same, since the Applicant has shown that a DNA amplification reaction can be performed at the surface of a PTC material. This could be particularly advantageous in providing a simpler device, with a reduced number of components, and indeed potentially enable more rapid amplification since there would be no need to heat the reaction vessel, since the vessel and heating element are one and the same. PTC can be moulded to produce reaction vessels of specific shapes and sizes, and thus the possibility of providing for small heating elements, and the design of simpler and more practicable integrated devices. In one embodiment one or more reagents for amplification could be freeze dried to the surface of the PTC reaction vessel, enabling a reaction to be performed for example simply by addition of water or a suitable buffer, plus the target nucleic acid.

The present invention will now be described with reference to the following non-limiting examples and drawings in which:

Figure 1 A is a graph representing the temperature readings inside a SmartCycler tube housed within the heating device. The heating device was placed inside a temperature controlled incubator set to

5, 10, 15, 20, 25, 30 and 35 °C and a constant 12 V was applied to heat the SmartCycler tube. The shaded area of the graph denotes the working temperature range of the BG LAMP assay;

Figure 1B is a graph representing the change in resistance as the PTC element is heated using a 12 V bench top power supply; and

Figure 2 is two graphs representing the temperature readings recorded inside the heating rig attached to a battery pack. The heating rig containing a SmartCycler tube filled with water was placed inside a temperature controlled incubator set to either 10 or 20 °C. Eight 1.5 V batteries in series were used to power the heating rig. The temperature profile achieved when the rig was powered by a 12 V bench top power supply is shown for comparison.

Examples

A heating rig/device was designed to include a PTC heating element (ceramic PTC thermistor) and used to thermostatically control an isothermal amplification reaction to specifically detect *Bacillus atrophaeus* genomic DNA. The PTC thermistors in the rig were able to heat and maintain a constant temperature in a reaction tube across a wide range of ambient temperatures using either a bench top or battery power supply.

Materials and Methods

Single sided PTC heating elements, part No. PS425C050S102H, were obtained from Midas Components Limited, Electra House, 32 Southtown Road, Great Yarmouth, Norfolk, NR31 0DU. These were selected based on a specified transition temperature (the temperature at which point resistance will increase) of 50 °C.

Master mixes for Loop mediated isothermal amplification (LAMP) were obtained from Optigene Ltd.

LAMP primers and probes specific to *Bacillus atrophaeus* (BG) were obtained from ATDbio Ltd. Southampton, UK. The primer and probe sequences used were:

BIP: 5'-TTGTCCAGCAGCTGATTGTTGGTTTTGGTCAGTTTGGTACAGAATTTGC-3';

FIP: 5'-GGACAATTCGGCACTGAATTCGCTTTTTGTTTCAGCAGATTGGTTTTGC-3';

LOOP B: 5'-Biotin-TGTTGTACGTTTGTTCGC-3'

LOOP F: 5'-FAM-GTGAAACTGATGCTCAGC-3'

B3: 5'-GCTCAACAAGTAAGAAAACA-3'

F3: 5'-TTTTCTGTTTCAGTGATTAGC-3'

A prototype heating rig was assembled from two PTC heating elements connected in parallel arranged on either side of a 50uL Cepheid SmartCycler reaction tube. The tube and associated heating elements were then coated in a release agent and the whole assembly was then fitted within a plastic box (45mm x 25mm x 14mm) and filled with an epoxy resin. Once the resin had set the SmartCycler tube was removed to create a heating chamber.

The PTC elements in the rig were connected to either a single output bench power supply unit (Thandar TS3021S) or a battery pack containing eight 1.5 V Duracell® Procell batteries. The temperature of the sample inside the reaction chamber was measured using a thermometer data logger (ExTech Easyview 15) fitted with a K-type bead wire temperature probe.

Thermal profiling

In experiments that measured the internal temperature of the SmartCycler tube at different ambient temperatures the rig was placed inside a temperature controlled incubator (INNOVA 44, New Brunswick Scientific). The temperature in the incubator was varied over a range of temperatures from 5-35C.

Isothermal DNA amplification reactions

Loop mediated isothermal amplification (LAMP) was chosen as the DNA amplification chemistry to demonstrate utility of the heating rig, due to its temperature profile matching the transition temperature of the PTC elements. Fifty microliter LAMP reactions containing 30uL mastermix, 10uL primer mix and 10uL of sample, containing 1pg of BG genomic DNA, were mixed prior to pipetting into the SmartCycler tube. The primer mix contained 2.4uM LAMP forward primer, 2.4uM LAMP reverse primer, 1.2uM LOOP forward primer, 1.2uM LOOP reverse primer, 0.6uM DISP forward primer and 0.6uM DISP reverse primer. In experiments to determine the working temperature range of the BG LAMP assay 25uL of reaction mix, containing 1pg of genomic DNA, was pipetted into 50uL thin walled reaction tubes and incubated in a heat block, at a range of temperatures between 45°C and 85°C for 20 minutes.

Detection of amplification products using gel electrophoresis and lateral flow strips

Reaction products were electrophoresed on a 2% agarose gel (Invitrogen G5018-02) and visualised with ethidium bromide staining. Specific reaction products from LAMP reactions were also detected by running on lateral flow test strips (BBI-Detection, Dundee). The lateral flow test strips contain gold nanoparticles conjugated to anti-FAM antibodies. Amplicon was diluted 1:10 with HEPES-Tween buffer and 100 µL diluted material loaded per strip.

Results**Working temperature range for the BG specific LAMP DNA Amplification chemistry**

BG specific amplification products were observed on agarose gels when reactions were performed at 55, 60, 65 and 70°C. Labelled products were also detected on lateral flow strips with the strongest positive test line seen when the reaction was run at 65°C. The working range of the assay was bounded by a lower temperature of 55°C and a higher temperature of 70°C.

Performance of the test rig at a range of ambient temperatures.

A SmartCycler tube containing 50 μ L of distilled water was inserted into the rig and a constant 12V was applied to heat the chamber. Having regard to Figure 1a, the temperature inside the tube was measured over a period of twenty minutes using a K-type temperature probe over a range of ambient temperatures from 5-35°C within a temperature controlled incubator. With a 12V constant supply the temperature of the liquid inside the SmartCycler tube rose rapidly over 2.5 min and then plateaued to within the working temperature of the BG specific LAMP assay. This thermal profile was achieved across a range of ambient temperatures from 5 to 35°C. Having regard to Figure 1b, during these measurements, the resistance of the PTC elements was also recorded to monitor the change in resistance with temperature

Performance of the LAMP assay in the test rig

The BG specific LAMP assay was performed in the test rig at a range of ambient temperatures. Twelve volts was applied to the heating rig and after each 20 minute reaction the amplification products were either electrophoresed on an agarose gel and visualised with ethidium bromides or detected using lateral flow test strips.

Demonstration using battery power

Having regard to Figure 2, the test rig was attached to a power pack containing eight 1.5 V batteries in series to demonstrate the potential of this system for hand held or disposable devices. Results show that from two different ambient temperatures, the batteries were able to deliver the same thermal profile as the bench top power pack.

A ceramic PTC thermistor has been used in combination with an isothermal DNA amplification chemistry to generate specific amplification products. The combination overcomes the significant problems of achieving thermostatic control of isothermal nucleic acid amplification reactions, especially for use in hand held devices.

Isothermal DNA amplification on the surface of a PTC chip

An isothermal DNA amplification reaction (recombinase polymerase amplification; RPA) was performed in a rig comprising a small plastic chamber glued to the surface of a PTC chip (single sided PTC heating elements, Midas Components Limited, UK; part no. PS425C050S102H). The RPA reaction was in direct contact with the surface of the PTC. The amplification reaction was conducted for 20 minutes at a reaction temperature of 41°C. The ambient temperature was approximately 20°C, and the reaction mixture, heated by the PTC, took one minute to reach the reaction temperature. A control reaction in a heating block at 37°C was also conducted for 20 minutes. Results showed that 2 pg of genomic DNA from BG could be amplified both in the PTC and control reaction, and then specifically detected on lateral flow test strips. Amplification of 200 fg of genomic DNA for BG was on the limit of sensitivity for the PTC heated reaction (as detected on lateral flow test strips), but that the sensitivity of the assay was increased by performing the reaction on a plasma coated PTC chip (as coated by P2i Ltd). The control reaction successfully amplified 200 fg of genomic DNA from BG, as detected on lateral flow test strips.

The PTC heating rig used in this study had a volume of approximately 6 cm³ for one reaction and this included associated insulation. Direct amplification on the PTC chip has been exemplified by the Applicant by performing an isothermal DNA amplification reaction at the surface of the PTC material itself, which lends itself to even smaller devices, such as approximately 0.25 cm³ for one reaction including insulation. Such small heating elements will enable the design of simpler more practicable integrated devices.

Claims

1. Method for heating a chemical, biological, and/or enzymatic reaction at a pre-determined temperature comprising use of a positive temperature coefficient heating element to control and maintain the temperature at the pre-determined temperature.
2. Method according to Claim 1, wherein the reaction is an isothermal nucleic acid amplification reaction.
3. Method according to Claim 1 or Claim 2, wherein the pre-determined temperature is capable of being maintained in ambient temperatures of between 5°C and 35°C.
4. A device for detection of nucleic acids using isothermal nucleic acid amplification comprising a positive temperature coefficient heating element.
5. Device according to Claim 4, wherein the device is hand-held.
6. Device according to Claims 4 and 5, wherein the device is disposable.

Figure 1

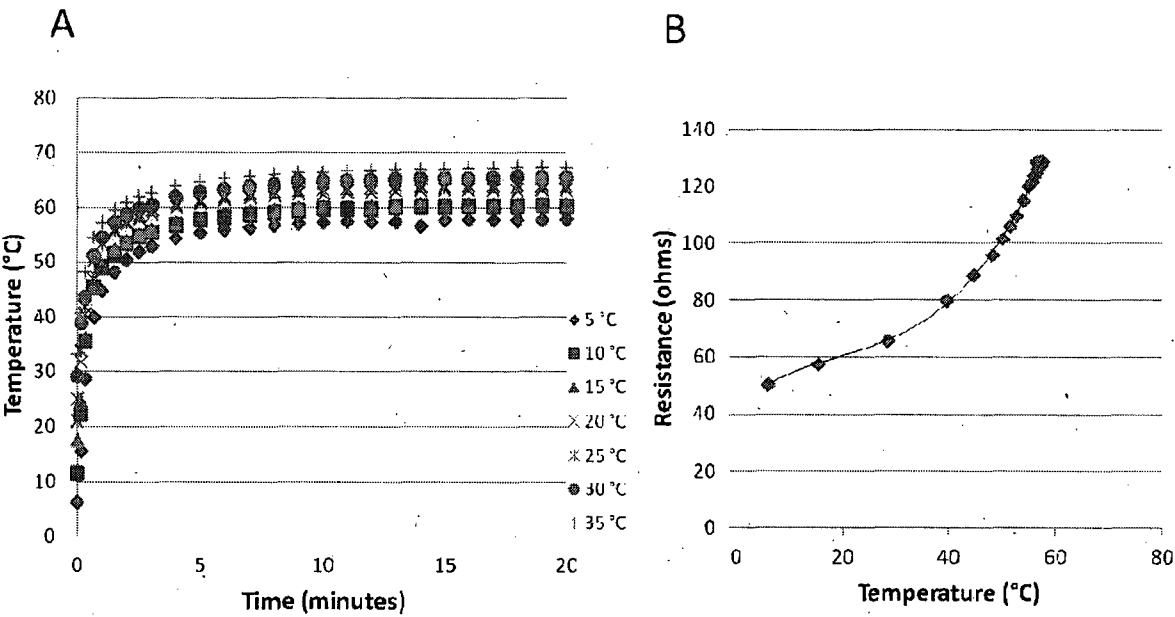
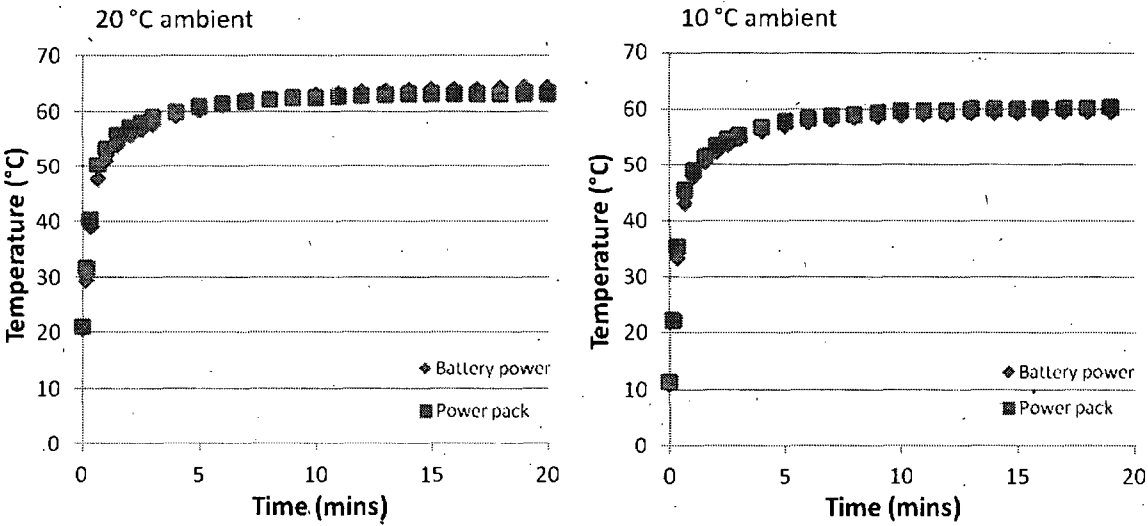


Figure 2



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/000026

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L7/00 G01N27/447 G01N30/30
ADD.

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)
B01L G01N

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 practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/028587 A1 (BAER THOMAS M [US] ET AL) 10 February 2005 (2005-02-10) paragraphs [0002], [0004], [0037], [0043] - [0045], [0049], [0055] - [0060], [0067]; figures 1-11 -----	1-6
X	US 6 548 788 B2 (KELLOGG GREGORY [US] ET AL) 15 April 2003 (2003-04-15) column 31, lines 7-43 column 36, lines 37-53 column 53, line 10 - column 54, line 12; figures 29-34 -----	1-6
X	JP S62 151760 A (TOSHIBA CORP) 6 July 1987 (1987-07-06) abstract; figures 1,2 ----- -/-	1-6



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

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Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Viskanic, Martino

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/000026

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2004/096819 A1 (MCMILLAN WILLIAM A [US] ET AL) 20 May 2004 (2004-05-20) paragraphs [0011], [0119], [0120], [0204]; figures 6,8,24 -----	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2015/000026

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
US 2005028587	A1	10-02-2005	US	2005028587	A1	10-02-2005
			US	2006191887	A1	31-08-2006

US 6548788	B2	15-04-2003	AU	7591998	A	11-12-1998
			EP	0983504	A2	08-03-2000
			JP	3469585	B2	25-11-2003
			JP	3537813	B2	14-06-2004
			JP	3725834	B2	14-12-2005
			JP	2000514928	A	07-11-2000
			JP	2003028883	A	29-01-2003
			JP	2003270252	A	25-09-2003
			JP	2005326432	A	24-11-2005
			US	6063589	A	16-05-2000
			US	6302134	B1	16-10-2001
			US	2001001060	A1	10-05-2001
			US	2002027133	A1	07-03-2002
			US	2003195106	A1	16-10-2003
			US	2004191125	A1	30-09-2004
			WO	9853311	A2	26-11-1998

JP S62151760	A	06-07-1987	NONE			

US 2004096819	A1	20-05-2004	AU	5929001	A	12-11-2001
			US	6783934	B1	31-08-2004
			US	2002031768	A1	14-03-2002
			US	2002034745	A1	21-03-2002
			US	2002034746	A1	21-03-2002
			US	2002058282	A1	16-05-2002
			US	2004096819	A1	20-05-2004
			US	2005255516	A1	17-11-2005
			US	2006014200	A1	19-01-2006
			WO	0184463	A2	08-11-2001
