Oct. 12, 1965 T. H. BENZINGER 3,211,531

MINIATURIZED REACTION VESSEL

Filed Jan. 21, 1963

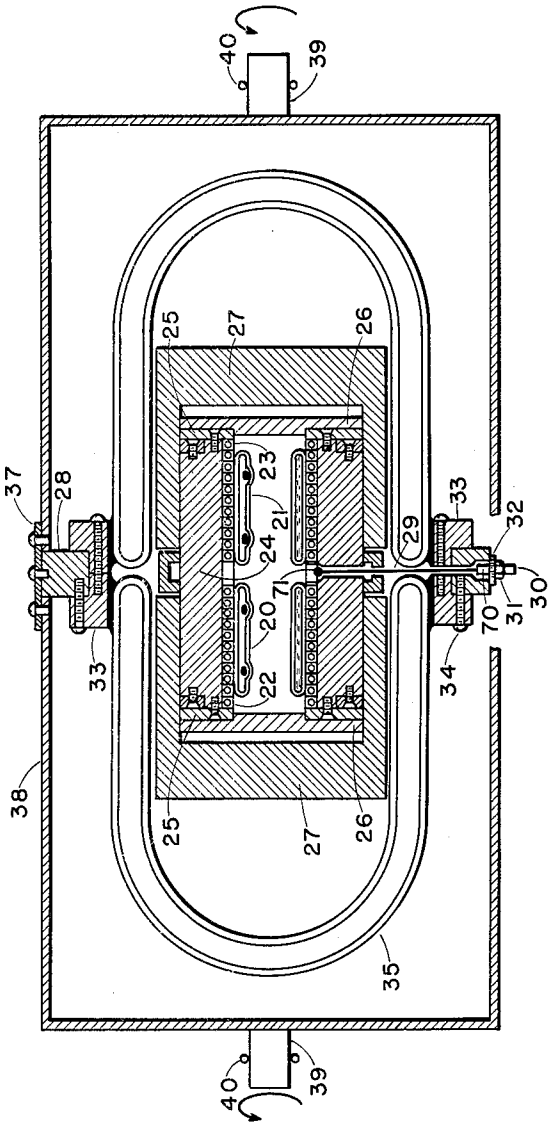


FIG. 1

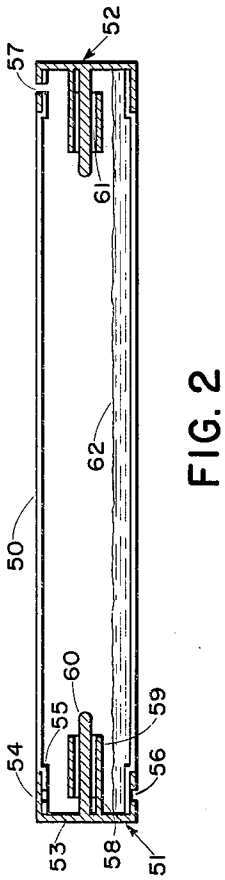


FIG. 2

INVENTOR.
Theodor H. Benzinger
BY
Attorney

United States Patent Office 3,211,531 Patented Oct. 12, 1965

3,211,531

MINIATURIZED REACTION VESSEL

Theodor H. Benzinger, Chevy Chase, Md., assignor to the United States of America as represented by the Secretary of the Navy

Filed Jan. 21, 1963, Ser. No. 253,008
8 Claims. (Cl. 23—259)
(Granted under Title 35, U.S. Code (1952), sec. 266)

The invention described herein may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment of any royalties thereon or therefor.

The present invention relates generally to calorimetric apparatus and procedures and, more particularly, to an improved reaction vessel for investigating the heat producing characteristics of reactions involving materials in suspension and in solution.

In applicant's copending application, Serial No. 17,232, filed March 23, 1960, there is disclosed a heatburst microcalorimeter whose response time and sensitivity make it possible for the first time to investigate quantitatively certain chemical and biochemical reactions. In this instrument the reactants are widely dispersed so as to maximize the heat transfer surface area, and a so-called "area thermopile," having its hot and cold junctions blanketing this surface and a confronting heat sink surface, respectively, performs both as a low thermal impedance heat transfer means and a highly efficient electrical signal generating device. Because of the relationship and coaction between the reaction surface, the thermopiles and the heat sink, the heat of reaction is rapidly discharged into the heat sink as a heat pulse, and the passage of this pulse through the thermopile imparts to the output voltage signal a pulse configuration. As a consequence of this mode of operation, which for the first time fully exploits the thermal potential difference between the reaction and the heat sink, the microcalorimeter of the above application has an increased sensitivity to instantaneous reactions, an improved speed of response to changing continuous rates of heat flow and a reduced error from inertial distortions and external temperature disturbances.

Moreover, to eliminate the need for mechanical mixing and stirring devices, a source of unknown error in the prior art equipment, the heatburst microcalorimeter of the above application and its reaction vessels are constructed and mounted so that only gravitational forces are used for carrying out these operations. Because of the enhanced power of resolution of this instrument, even the relatively small amount of heat introduced by this method of mixing can be accurately ascertained by repeating the original mixing motions after thermal equilibrium has been established and recording the voltage wave form so generated.

One of the major factors influencing the construction of the reaction vessel and the blank vessel comes from the previous stated desideratum that the reaction material present the largest obtainable surface area to the area thermopile for accelerating the heat flow therefrom into the heat sink. To achieve this dispersal, applicant's copending application, Serial No. 17,232, employs reaction vessels wherein the bulk material is accommodated between concentric, cylindrical walls, with the other reactants isolated therefrom prior to mixing by either a longitudinal dividing wall, a circular transverse wall, drop wells or ring-type dropholders. However, in these vessels reactants cannot be kept separated during rotating motion. Therefore, for experiments involving living material, such as, for example, bacteria or cell cultures, a test tube type reaction vessel for continuous rotation,

closed off at each end by a cap having a dome-shaped compartment projecting from the inner wall thereof, is utilized. Each of these compartments has an aperture formed at its apex. With this design, different substances can be accommodated in each compartment, and these substances can be kept separated during rotating motions and yet be mixed, though only incompletely, at predetermined times with the bulk suspension by tilting. Thus, one holder may, for example, add virus to a cell culture while the other may add an enzyme poison to stop all metabolic activity and establish the zero base level of the system at the end of a particular recording, rotating operation.

Most biological materials, with the exceptions of solutions of molecular particles, consist of matter in suspension rather than in solution. In this category are, for example, microorganisms, parasites, protozoa, eggs, sperms, bacteria, virus, tissue slices, cells, cell cultures, homogenates, subcellular particles, such as nuclei, spindles, mitochondria, microsomes. With many of these objects, the heat production and oxygen consumption depend upon the rate of diffusion of oxygen to the often sedimented and tightly packed particles. Consequently, the reaction vessels accommodating these objects must be rotated during the investigation. Because of this mode of operation, some provision must be made in the vessel for keeping two or three reactants separated during periods of such continuous rotation.

With some of the reaction vessels disclosed in copending application, Serial No. 17,232, some difficulty has been experienced in rinsing out the reactants and insuring that each drop thereof mixes with the bulk solution accommodated in the main body portion of the vessel. As a result, these substances have to be inserted stoichiometrically in excess of the bulk solution. This, of course, complicates the data reduction operation.

These difficulties are overcome by the invention which is based on a discovery used also in copending application, Serial No. 253,007, filed January 21, 1963, for the miniaturized reaction vessel, namely, that liquid materials flow easily in, and may be easily rinsed out from, narrow annular spaces between two cylindrical surfaces.

It is accordingly a primary object of the present invention to provide an improved reaction vessel for a rotating microcalorimetric apparatus and for procedures with materials in suspension rather than solution.

Another object of the present invention is to provide a reaction vessel for microcalorimetry which accommodates a bulk solution of one and smaller quantities of two other, different reactants.

A still further object of the present invention is to provide a reaction vessel wherein certain reactants are accommodated in annular spaces between two cylindrical walls.

A yet still further object of the present invention is to provide a reaction vessel wherein various reactants are kept separate from suspended material during rotation and are mixed therewith upon tilting.

A yet still further object of the present invention is to provide an improved reaction vessel wherein the reactants are accommodated within a combination cap and dropholder.

A still further object of the present invention is to provide a reaction vessel of simple design which is easy to assemble, disassemble and clean.

Other objects and many of the attendant advantages of this invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIG. 1 illustrates a heatburst microcalorimeter of the

type disclosed in applicant's copending application, Serial No. 17,232; and

FIG. 2 is one embodiment of a reaction vessel constructed according to the present invention.

Referring now to FIG. 1 of the drawings, the heat-burst microcalorimeter in the twin calorimeter configuration is seen to comprise a double-walled reaction vessel **20** and a blank vessel **21** disposed in an end-to-end relationship within a cylindrical heat conducting sleeve whose outer surface is completely blanketed by the hot junctions of a pair of "area thermopiles" **22** and **23**. Each of these so-called "area thermopiles" is fabricated by first winding a constantan conductor in a helix, then copperplating half of each individual turn thereof to form thermojunctions at diametrically opposite points, and thereafter coiling the plated helical structure about the outer surface of the cylindrical sleeve. Surrounding both area thermopiles is a cylindrical heat sink **24** whose inner surface is completely blanketed with the cold junctions of these thermopiles. This heat sink is closed off by metal covers **26** and also by heavy metallic caps **27**, the latter serving to increase the heat capacity of the system and provide a thermal short circuit between the various arcuated sectors forming the heat sink. The above assembly, which is isolated from the local environment by a pair of confronting U-shaped De War vessels **35**, is held in radial suspension to further restrict heat inroads into the temperature sensitive portion of the instrument by means of wires **29** which are fastened to ring **28** and heat sink **24**. Also secured to this suspension ring are a pair of closure members **38**, and the space between these members and the De War vessels is filled with an insulating material, such as styrofoam, for additional thermal protection. A suitable pair of shafts **39** attached to opposite ends of these closure members permit the complete instrument to be rotated about its longitudinal axis for mixing and other allied purposes such as preventing sedimentation of suspended particles.

In investigating the possible use of capillary devices for retaining quantities of reactant, it was discovered that liquids and gases tend to flow more easily in narrow, annular spaces between cylindrical surfaces than in open capillaries. For example, the flow in a one millimeter annular space was found to be better than that in an open capillary of three millimeter diameter. For best flow results, it was experimentally determined that the inner diameter of the annulus should preferably exceed 1.25 mm.

Referring now to FIG. 2, it will be seen that the reaction vessel of the present invention embodying this discovery, in one modification, consists of a test-tubelike tubular member **50**, closed off at each end with combination cap and dropholders **51** and **52**. Each cap has an end wall portion **53** and a circular flange **54** which is adapted to fit over the reduced diameter end portion **55** and form an airtight seal therewith. By reducing the diameter of member **50** at each end by approximately the thickness of this flange, the assembled vessel presents an uninterrupted, cylindrical shape to the cylindrical sleeve of the calorimeter into which it fits. Matching holes **56** and **57** are drilled through both caps and the reduced diameter portion of member **50**. The apertures permit air to escape from the reaction vessel when the apparatus is assembled. Also, these apertures provide access to the interior of the vessel so that, for example, the nose of a conventional fluid dropper can be inserted therethrough during the bulk fluid filling operation.

Supported from the iner wall of each cap by a stem or stalk **58** is a capillary or tubular element **59** which has concentrically disposed therein a cylindrical core **60**. This core extends a slight distance beyond the top rim of capillary **59** and is rounded off to prevent any of the reactant from adhering thereto. From what has been said hereinbefore, it will be appreciated that the annular space **61** defined by the inner wall of capillary **59** and the outer wall of core **60** serves as the temporary retainer for the reactant.

When measured droplets of reactants are placed in these annular spaces, capillary action prevents the material from flowing out even when the vessel is tilted into a perpendicular position. As long as the reactant vessel is kept in a more or less horizontal position when filled, the bulk liquid **62**, with suspended material in its main space, will remain isolated from the various reactants. And this isolation of the first reactant persists even when the vessel is rotated abouts its longitudinal axis. However, when the vessel is tilted upwardly, for example, the second reactant in the lower well will be almost instantaneously washed out as the bulk liquid first splashes against the lower end wall and then rises through the annular space between the capillary and its central core. Likewise, and first when the vessel is tilted in an opposite direction, the bulk liquid will wash out the third reactant housed within the opposite well.

It will be appreciated that the stalks or stems supporting the capillary tubes should be of minimum thickness so as not to interfere too much with the entrance of the liquid into the annular space.

Since the apparatus of FIG. 2 comes apart easily at the end caps, the assembly, disassembly and cleaning of the vessel present no problems. Likewise, since the filling of the wells is done with the caps detached, this operation can be performed without difficulty and with a high degree of precision.

To prepare the apparatus for use, each of the annular spaces is filled by a fluid dropper. The closure caps are then fitted to the open ends of the reaction vessel and rotated to align the matching holes **56** and **57**. One of these apertures may now be employed as a filling aperture and a needle-nose dropper inserted therethrough into the main body of the reaction vessel for introducing the bulk fluid **62**. These holes can thereafter be closed by simply twisting each cap in its cylindrical grease joint and, after thermal equilibrium has been obtained, the reaction vessel may be placed within the core of the calorimeter.

The vessels constructed according to the modification of FIG. 2 may be fabricated to accommodate approximately four milliliters of bulk solution and one hundred to two hundred microliters of reactants in the wells. They may be made of glass with carbon molding techniques to hold the tolerances of the cap joints, or of gold-laminated stainless steel with solid gold caps. And by designing them with a relatively small diameter, for example, approximately fifteen millimeters, the end losses of heat mentioned above can be reduced to an acceptable level.

While the concept of temporarily storing relatively small quantities of fluids or materials in annular spaces and washing them out by or mixing them with another fluid which is caused to flow through these spaces is disclosed in connection with calorimetry instruments and the like, it will, of course, be appreciated that this technique is of a generic nature and may be used in other chemical or fluid mixing and stirring applications.

It would also be mentioned that while the capillary **59** and its core **60** are mounted along the axis of symmetry of tubular element **50**, these structures can, of course, be displaced from this location to suit the particular conditions prevailing. Also, the cross-sectional shape of the capillary and its core can take various forms other than that illustrated in FIG. 2.

It would also be pointed out that the reaction vessel can be formed with an integral closure piece so that only one end cap need be used. Of course, this would mean that only one reaction substance would be available to coact with the bulk solution. It would also be pointed out that in the modification of FIG. 2 the bulk solution is placed in the main body of the vessel only after both of the end caps are in place for obvious reasons. Because of the reduced diameter portions of the reaction vessel adja-

cent each end thereof, a shallow well is created in the main body of the tube, and this well can accommodate a finite amount of bulk fluid. Thus, if desired, the bulk fluid can be introduced into this well when the vessel is in a horizontal attitude with either or both end caps removed.

Obviously many modifications and variations of the present invention are possible in the light of the above teachings. It is therefore to be understood that within the scope of the appended claims the invention may be practiced otherwise than as specifically described.

What is claimed is:

1. For use with a reaction vessel having one end open, the combination of

a closure cap having an end wall portion,

a sleeve supported from said end wall and spaced therefrom,

said sleeve occupying a position within the main body of said reaction vessel when said closure cap is secured to the open end of said reaction vessel,

and a rod retained within said sleeve with its longitudinal axis parallel to the axis of symmetry of said sleeve,

the separation between the inner wall surface of said sleeve and the outer wall surface of said rod being such that a fluid substance placed therebetween is retained therein by capillary action.

2. In an arrangement as defined in claim 1 wherein said rod is concentrically positioned within said sleeve and extends the complete length thereof.

3. In an arrangement as defined in claim 2 wherein said rod is supported from the end wall portion of said closure cap.

4. A combined closure cap and interim fluid storage device for an open-ended reaction vessel comprising,

a member having an end wall portion and a rim portion projecting therefrom,

a sleeve supported away from said end wall portion with its longitudinal axis perpendicular to said end wall portion,

a core supported from said end wall and extending the length of said sleeve,

the separation between the inner wall surface of said sleeve and the outer surface of said core being such that a fluid substance disposed therebetween is maintained therein by capillary action.

5. In combination, a tubular element,

said tubular element having a reduced outer diameter portion adjacent each end thereof,

a removable closure cap sealing off each open end of said tubular element and making said tubular element fluidtight,

each closure cap having an end wall portion and a circular rim portion,

the inner diameter of said rim portion being slightly greater than the outer diameter of the reduced diameter portion of said tubular element,

and the outer diameter of said rim portion being equal to the unreduced outer diameter of said tubular element.

each rim portion and each reduced diameter portion having an aperture cut therethrough,

the aperture in each rim portion and reduced diameter portion being aligned when said closure cap is in place,

a sleeve secured to the end wall portion of each closure cap in a spaced relationship therefrom,

a core retained within each sleeve, the longitudinal axis of each core coinciding with the longitudinal axis of symmetry of each sleeve.

6. A reaction vessel for use in the core of a calorimeter of the type wherein only gravitational forces are employed to intermix the reactants comprising

a tubular member;

means closing off the ends of said member and forming with said member a fluidtight enclosure;

a sleeve;

means for supporting said sleeve within said fluidtight enclosure such that a flow of fluid can take place into one end of said sleeve, through said sleeve and out the other end thereof;

a core concentrically retained within said sleeve, the space between said core and the inner wall of said sleeve serving as an open-ended annular compartment for retaining therein by capillary action a fluid reactant.

7. A reaction vessel for use in the core of a calorimeter of the type wherein only gravitational forces are employed to intermix the reactants comprising

a tubular member;

a detachable closure element sealing off each end of said member and forming with said member a fluidtight enclosure;

a pair of sleeves;

means connected to each closure element for supporting one sleeve of said pair of sleeves within said fluidtight enclosure such that a flow of fluid can take place into one end of that sleeve, through that sleeve and out the other end thereof; and

a core concentrically disposed within each sleeve, the space between the inner wall surface of each sleeve and each core being such that any reactant fluid placed therein is retained there by capillary action.

8. In an arrangement as defined in claim 7 wherein each core consists of a cylindrical member which has a diameter of at least 1.25 millimeters.

References Cited by the Examiner

UNITED STATES PATENTS

1,482,966	2/24	Bevan	23—253
1,594,370	8/26	Kubota	23—253
2,052,185	8/36	Lewis	73—382

MORRIS O. WOLK, *Primary Examiner.*

JAMES H. TAYMAN, JR., *Examiner.*