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STUDYING DISPERSOID SYSTEMS
Method of introducing an injecting needle into calorimetric chamber of capillary titration calorimeter

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Measurements of energy transformation in mitochondria are done on a capillary differential titration calorimeter CTD2156. It is important to mention that a sediment is quickly formed by the mitochondria suspension without mixing by means of a vibrating needle. During the measurements, the vibrating needle is located inside the working volume of the chamber. The design of the calorimeter is substantiated theoretically. It provides a new mode of a reagent input in the measuring volume of the calorimetric chambers. It expands the spectrum of tasks that can be solved using this instrument. In the capillary calorimeter the calorimetric chambers unit is simple and small in size. These advantages of capillary chambers provide an opportunity to unite 20 capillary calorimetric chambers in one calorimetric block. It allows designing a multi-channel titration calorimeter. There are obvious advantages of such a calorimeter over other instruments in screening researches and in researches of objects maintaining stability only for a short time.

Keywords: *capillary calorimetric chamber, differential titration calorimeter, heat-conducting bridge, mitochondria, mixing reagents in a capillary chamber, sensitive volume of calorimetric chambers*

Introduction

Many systems are known to form a sediment, for example, the suspension containing mitochondria. At research of such systems it is important to ensure mixing of the sample studied. In the CTD2156 capillary titration calorimeter the calorimetric chambers are located horizontally. The mixing is achieved by introducing the mitochondria suspension into the calorimetric chamber by an injecting vibrating needle [1]. This solves the problem of mixing the same samples in the capillary chamber. In known analogues with a vertical chamber this process is very complicated. The decision of this problem is urgent and promising, as it will be a new step in developing capillary titration calorimeters. Simple CTD2156 calorimetric chambers, their small size provide a possibility to design a multi-channel calorimeter. Such a calorimeter is rather promising for research of objects, which remain a stable only for a short time. This enables to run a set of about 20 different versions of the same experiment. Thus a multi-channel calorimeter can become an important tool for studying mitochondria and also in performing a number of physico-chemical researches requiring a complete and exact thermodynamic description of binding processes.

Brief description of a capillary titration calorimeter

A capillary titration calorimeter comprises a calorimetric unit, a titration unit, an electronic unit, a nanovoltmeter HP34420A, a computer with two boards Lab-PC+ and a PC-TIO-10 board (all boards are made by National Instruments, USA). The calorimetric unit comprises two calorimetric cells designed as capillary tubes. To separate the sensitive volume, an active and a passive heat-conducting bridges are used. The heat-conducting bridge 1 is designed to separate the sensitive volume of calorimetric cells. The heat exchange with the environment through the outlet part of the capillary tubes is prevented by the heat-conducting bridge 2.

Figure 1 shows the calorimetric unit. The main elements of the thermostatic control in the calorimetric cells are a constant-temperature shield and a thermopile-based thermostat. The housing of the instrument represents a load-carrying structure bearing a calorimetric unit and a titration unit. The titration unit consists of two syringes located in a common case. The pistons of the dispersing syringes are joined together by means of a connecting bar. To perform the titrant injection process in the calorimetric cells, the case of the syringes is connected to a screw mechanism, the bar

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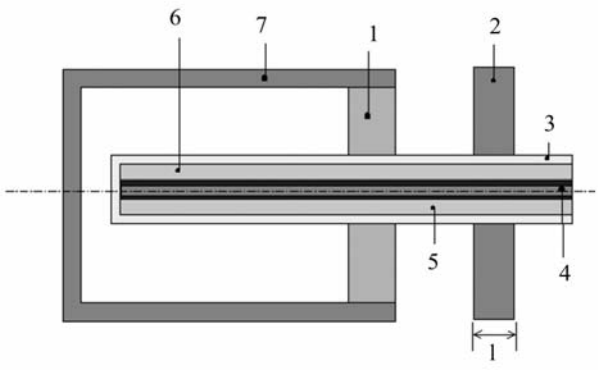

Fig. 1 Calorimetric unit ensuring the mode of introducing an injecting needle into the calorimetric chamber through the active bridge: 1 – bridge 1 (passive), 2 – bridge 2 (active), 3 – chamber, 4 – needle with titrant, 5 – sample, 6 – reaction area, 7 – shield

being connected to the other screw mechanism. The screw mechanisms are triggered by stepping motors. Such a design provides for computer control of the calorimeter, data acquisition and processing, etc. A more detailed description of the instrument is given in [1, 2].

Mode of introducing an injecting needle into the calorimetric chamber through the active bridge

The design of the instrument ensuring such an input of the injecting needle in the calorimetric chamber is substantiated theoretically. The calculations have been given for the calorimetric unit shown in Fig. 1.

The equal temperature of thermal bridge 2 T_{br2} and shield T_{sh} is provided with the automated control system. The heat capacity of bridge 2 is much greater than the heat capacity of the part l of the needle. The needle, filled with a reagent, is moved from the top to the bottom of the chamber with the speed υ. The initial temperature of the needle is T_{in}, $T_{in}>T_{br2}$. The part l of the needle will have temperature T_{end} after its movement through bridge 2. Thus this part of the needle loses heat at its movement through bridge 2:

$$Q=C(T_{in}-T_{end}) \quad (1)$$

where C is the heat capacity of the part l of the needle.

The heat transfer from the needle to bridge 2 is realized via heat-conductivity and convection through the liquid between the needle and the wall of the chamber. The thermal contact of the chamber with bridge 2 is considered to be ideal. Therefore the chamber temperature is equal to that of thermal bridge 2 in the range of their contact. Thus

$$Q=\alpha(\bar{T}-T_{br2})At \quad (2)$$

where α is the heat transfer coefficient from the needle to bridge 2 with height l; A is the area of contact of the needle with thermal bridge 2; $t=l/\upsilon$ is the time of moving the needle part through thermal bridge 2; $\bar{T}$ is the average temperature of the needle part in thermal bridge 2.

Setting Eq. (1) to be equal to Eq. (2), we obtain

$$C(T_{in}-T_{end})=\alpha(\bar{T}-T_{br2})At$$

where $\bar{T}\approx(T_{in}+T_{end})/2$. By substituting $\bar{T}$ in the previous expression we obtain:

$$C(T_{in}-T_{end})=\alpha\left(\frac{T_{in}+T_{end}}{2}-T_{br2}\right)At \quad (3)$$

Denoting $\Delta T=T_{in}-T_{br2}$ to be the overheating of the needle with a reagent relative to bridge 2 on the input of thermal bridge 2; $\delta T=T_{end}-T_{br2}$ is overheating of the needle with a reagent relative to bridge 2 on the output of thermal bridge 2.

By substituting ΔT and δT in Eq. (3) we obtain:

$$2C(\Delta T-\delta T)=\alpha(\Delta T+\delta T)At$$

After transformation of this expression we obtain

$$\delta T=\frac{\left(2C-\alpha A\frac{l}{\upsilon}\right)\Delta T}{2C+\alpha A\frac{l}{\upsilon}} \quad (4)$$

So long as here is a transfer of heat by heat-conductivity and by convection from the needle to bridge 2, we have $\delta T\geq 0$ and

$$C\Delta T\geq\frac{\alpha Al\Delta T}{2\upsilon}$$

Thus, the numerator in Eq. (4) is positive or is equal to zero with

$$C=\frac{\alpha At}{2\upsilon} \quad (5)$$

For given l the critical moving speed of the needle with a reagent can be determined from Eq. (5)

$$\upsilon_{cr}=\frac{\alpha Al}{2C} \quad (6)$$

With $\upsilon<\upsilon_{cr}$ the temperature of the needle with a reagent becomes equal to the temperature of thermal bridge 2 up to the moment of its exit from bridge 2.

With $\upsilon>\upsilon_{cr}$ the temperature of the needle with a reagent on an output of thermal bridge 2 differs from that of thermal bridge 2 by δT.

The efficiency of thermal bridge 2 is reduced at increasing the moving speed of the needle with a reagent. This follows from Eq. (4) with $\upsilon\rightarrow\infty$, $\delta T\rightarrow\Delta T$.

It is applicable also when the initial temperature of the needle is less than the temperature of thermal bridge 2.

Equations (4) and (6) contain factor α which is the heat transfer coefficient. The α factor is estimated from the average liquid speed (υ_{liq}) in a gap between the internal chamber surface and the needle, which is evaluated as

$$\upsilon_{liq} = \frac{\upsilon}{2} - \frac{\upsilon}{\left(\frac{r_{chamber}^2}{r_{n1}^2} - 1\right)}$$

where υ is the needle moving speed; $r_{chamber}$ is the inner chamber radius; r_{n1} is the outer needle radius. For $r_{n1}=0.15\cdot10^{-3}$ m and $r_{chamber}=0.6\cdot10^{-3}$ m, υ_{liq} is 0.433 υ. Here both the size of the radial gap and the liquid displacement speed from the chamber during introducing the needle into it are taken into account. In the gap the liquid movement is laminar since in this case the Reynolds number is less than 2000. For the laminar liquid flow in a round channel with

$$Pe\frac{d}{l} < 12 \quad (7)$$

the Nusselt criterion is known [3] not to depend on the liquid flow speed (υ_{liq}) and to be equal to

$$Nu = \frac{\alpha d}{\lambda_{liq}} = 3.66 \quad (8)$$

where *Pe* is the Peclet thermal similarity criterion; $d = 2\sqrt{r_{chamber}^2 - r_{n1}^2}$ is the equivalent channel diameter; λ_{liq} is the thermal conductivity factor of the liquid. In this case *Pe* is

$$Pe = \frac{\upsilon_{liq} d}{a_{liq}} \quad (9)$$

where a_{liq} is the thermal diffusivity factor of the liquid.

The value of the critical heat transfer factor (α_{cr}) obtained from Eq. (8) is equal to

$$\alpha_{cr} = \frac{Nu\lambda_{liq}}{d} = 1956\ \mathrm{W\ m^{-2}\ K^{-1}}$$

where *Nu*=3.66, $d=1.16\cdot10^{-3}$ m, the thermal conductivity factor of water $\lambda_{liq}=0.62$ W m^{-1} K^{-1} at 30°C.

Substituting Eq. (9) in Eq. (7) we obtain the liquid speed (υ_{liq}), when α_{cr} does not depend on it,

$$\upsilon_{liq} \le \frac{12la_{liq}}{d^2} \le 10.7\cdot10^{-3}\ \mathrm{m\,c^{-1}}$$

where the thermal diffusivity factor of water at 30°C is $a_{liq}=0.15\cdot10^{-6}$ m^2 c^{-1}.

Hence the needle moving speed (υ) is less than $24.7\cdot10^{-3}$ m c^{-1}.

Thus the heat transfer factor (α_{cr}) being less than 1956 W m^{-2} K^{-1} does not depend on the needle moving speed (υ) and is constant, when υ is less than $24.7\cdot10^{-3}$ m c^{-1}. For the further calculation we take $\alpha=1000$ W m^{-2} K^{-1}, that is substantially smaller than α_{cr}. In [3] the α factor determination method used by us is described in more detail.

The forced liquid turbulization specified by the Reynolds number to be about 17 for water with temperature of 30°C is formed by radial vibration of the injecting-needle with the frequency of 30 Hz and the average amplitude at the needle length is $0.75\cdot10^{-3}$ m to provide an efficiency of the active bridge. In this case the heat transfer factor increases by about an order. Thus the above calculated heat transfer factor of 1000 W m^{-2} K^{-1} is suitable for all modes of an introducing needle.

To calculate υ_{cr} from Eq. (6) it is necessary to determine *C* and *A*.

The heat capacity of the needle volume with water at height *l* is:

$$C = \pi C_{p_{ss}} \rho_{ss} (r_{n1}^2 - r_{n2}^2) l + \\ + \pi C_{p_{H_2O}} (30°\mathrm{C}) \rho_{H_2O} (30°\mathrm{C}) r_{n2}^2 l$$

and

$$A = 2\pi r_{n1} l$$

where $C_{p_{ss}}$ is the isobaric specific heat capacity of stainless steel, ρ_{ss} is the density of stainless steel, r_{n1} is the external radius of the needle, r_{n2} is the internal radius of the needle.

With $C_{p_{ss}}=0.46\cdot10^3$ J kg^{-1} K^{-1}; $\rho_{ss}=7.9\cdot10^3$ kg m^{-3}; $C_{p_{H_2O}}=4.187\cdot10^3$ J kg^{-1} K^{-1}; $\rho_{H_2O}=0.996\cdot10^3$ kg m^{-3}; $r_{n1}=0.15\cdot10^{-3}$ m; $r_{n2}=0.1\cdot10^{-3}$ m; $l=8\cdot10^{-3}$ m the values $C\approx2.2\cdot10^{-3}$ J K^{-1} and $A=7.54\cdot10^{-6}$ m^2 are obtained.

Thus from Eq. (6) $\upsilon_{cr}=13.7$ mm s^{-1}.

The heat capacity of the liquid C_{liquid} filling the chamber between the needle and the wall of the chamber is involved in heat transfer. It is necessary to take into account the total heat capacity upon determining υ_{cr}. The total heat capacity is $C_{total}=C+C_{liquid}$, where

$$C_{liquid} = \pi C_{p_{H_2O}} (30°\mathrm{C}) \rho_{H_2O} (30°\mathrm{C}) (r_{chamber}^2 - r_{n1}^2) l$$

and $r_{chamber}$ is the internal radius of the chamber.

$C_{liquid}=35.4\cdot10^{-3}$ J K^{-1} is obtained for $r_{chamber}=0.6\cdot10^{-3}$ m.

Thus $C_{o\delta}=37.6\cdot10^{-3}$ J K^{-1} and $\upsilon_{cr}\approx0.8$ mm s^{-1}.

Hence the critical speed range (υ_{cr}) of CTD2156 varies from 0.8 to 13.7 mm s^{-1}.

The thermal effect from injection of the needle in the measured volume of the chamber does not exceed the noise level of the instrument. The energy transformation in mitochondria for $\upsilon=2$ mm s^{-1} is measured.

Measurement of energy transformation in mitochondria on capillary titration calorimeter

Chemicals

All reagens, unless otherwise specified, were obtained from United States Biochemical Corp. (Cleveland, Ohio, USA). ADF (adenosine diphosphate) was obtained from Sigma Chemical Co. (St. Louis, MO, USA); CCCP (carbonyl cyanide *m*-chlorphenylhydrazone), EGTA (ethylene glycol bis (β-aminoethyl ether) N,N'-tetraacetic acid), HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid), D-glutamic acid were from Serva Feinbiochemica GmbH (Heidelberg, Germany); Succinic acid from SynthVita Co. (Tula, Russia); Sucrose from Sigma-Aldrich Quimica (Madrid, Spain); potassium hydroxide from Lachema – Chemapol AG (Prague, Czech Republic). All chemicals were of the highest purity available. Bidistilled water was used for all preparations. In calorimetric measurements the medium (pH 7.4) contained 125 mM KCl, 3 mM KH_2PO_4, 1 mM $MgSO_4$, 10 mM HEPES, 0.25 mM EGTA, the substrate contained 5 mM potassium succinate, 0.25 mM potassium glutamate and phosphate acceptor was 90 μM ADP, and the uncoupler was 5 μM CCCP.

Preparation of mitochondria

Mitochondria were isolated from livers of Wistar rats weighing between 200 and 250 g by the method of differential centrifugation. The procedures of Johnson and Lardy [4] were followed. The isolation medium for rat liver mitochondria consisting of 0.3 M sucrose, 10 mM HEPES was adjusted to pH 7.4. The medium (pH 7.4) consisting of 150 mM sucrose, 125 mM KCl, 3 mM KH_2PO_4, 10 mM HEPES, 0.5 mM $MgSO_4$, 0.25 mM EGTA was used for mitochondria incubation. The mitochondrial protein concentration was 1 mg mL^{-1}.

Measurements of energy transformation in mitochondria

At present the given study trend is actively developed and is well presented in scientific publications. However by contemporary calorimeters one cannot be recorded the measured information on started process phase due to long transitional process which accompany a reaching of equilibrium state. This transitional process is continued some ten minutes but the time of measurement is practically unlimited [5–9]. The measurement of these processes on the capillary titration calorimeter allow a time of a transitional process to be decreased to a few seconds on unlimited duration of the whole of a measurement process. The CTD2156 operation mode with a vibrating needle has been presented earlier [1]. The measurements of the mitochondria energy transformation done on the calorimeter demonstrate the ability of the CTD2156 instrument. In the given experiment, the rat liver mitochondria were a research object. The calorimetric measurements were carried out at 26°C. The injecting needle had a mixing frequency of 20 Hz with an amplitude of 1 mm. The sample volume and injection volume were 156 and 2 μL, respectively. Before the experiment, the addition was stored in a refrigerator. The time between the uptake of the addition and its injecting into the chamber is about 60 s. For experiment 1 the calorimetric chamber contained the medium plus the substrate. For experiments 2 and 3 the chamber contained these plus ADP and these plus CCCP, respectively. The preparated rat liver mitochondria were injected.

The experimental data are given in Fig. 2. Curve 1 corresponds to the minimal speed of respiration. Mitochondria are in the environment without ADP, that interferes with their respiration. The initial site on curve 2 corresponds to the maximal speed of respiration. Mitochondria respiration is stimulated by a physiological addition of ADP. After consumption of ADP, the respiration again becomes minimal. Curve 3 corresponds to the transformation of all energy of respiration into the heat. The uncoupler permits the oxidation to proceed without phosphorylation. The barrier limiting the mitochondria respiration is eliminated due to the addition of CCCP to the environment. The maximal mitochondria respiration is reduced in the process of consumption of oxygen in the sample.

The given measurements were done in a mode of injecting the suspension of mitochondria into the calorimetric chamber using an injecting needle that

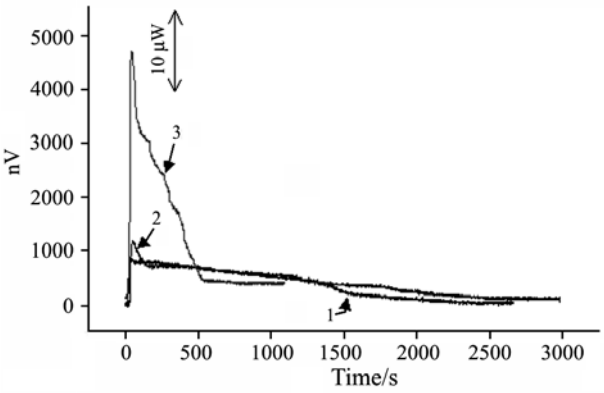

Fig. 2 Measurements of energy transformation in mitochondria. The recording of the heat production is a result of a slow rate of oxygen uptake in the presence of the substrate and absence of ADP, when 1 – 'tightly coupled' mitochondria are suspended in an isotonic buffer; 2 – the presence of ADP in the calorimetric chamber at the beginning of the process; 3 – the presence of the oxidative phosphorylation uncoupler (CCCP). The experimental conditions are described in the text

was moved from the top to the bottom of the chamber. During the measurements the injecting needle was located inside of the working volume of the chamber.

CTD2156 is the basis for designing multi-channel capillary differential titration calorimeter

The method of comparison of various titration calorimeters by the ability of the minimal energy measurement is proposed [2]. For this purpose the signal/noise ratio is used. This ratio allows the chamber volume and the measuring thermopile parameters to be optimized. For the semi-conductor thermopile and for the calorimetric chambers of a large volume it is possible to provide the maximum of the given ratio.

However in this case the calorimeter has some essential disadvantages such as: decreasing the stability of the semi-conductor thermopile in comparison with a metal thermopile, large sizes of chambers and others [1]. In this case it is impossible to investigate long-lasting processes. Moreover there are problems with mixing of reagents and separating the sensitive volume of calorimetric cells.

The calorimeter with capillary calorimetric chambers is devoid of such disadvantages. It should be noted that the instrument providing measurements of the minimal energy has conclusive advantages on the quality of mixing the reagents and on separating the sensitive volume of calorimetric cells [1, 2]. There is also an opportunity of studying dispersoid systems.

The high stability of experimental data received with such a calorimeter confirms high efficiency of the mode of introducting an injecting needle into the calorimetric chamber through the active bridge. The functioning of thermal bridges of the calorimeter has been also described in our paper [2].

The unit of calorimetric chambers of a capillary calorimeter (Fig. 1) is simple and small in size. These advantages of capillary chambers provide an opportunity to unite 20 capillary calorimetric chambers in one calorimetric block. In its turn this allows designing a multi-channel titration calorimeter. There are obvious advantages of such a calorimeter over other instruments in screening researches and in the researches of objects maintaining stability only for a short time.

Conclusions

The theoretical substantiation of new solutions of a calorimeter design is given. The proposed design provides a new mode of a reagent input into the measuring volume of the calorimetric chambers. Therefore the spectrum of tasks solved on this instrument is much wider. The CTD2156 titration calorimeter provides a precision measurement of the mitochondria energy transformation. The task of designing a multi-channel calorimeter is urgent. The multi-channel calorimeter can become an important tool for a number of physico-chemical researches requiring a complete and exact thermodynamic description of binding processes.

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