DMLM III DEVICE DEVELOPMENT FOR ENZYMES BIOCHEMICAL REACTIONS IN MICROGRAVITY

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ABSTRACT

The goal of this experiment was to investigate the invertase enzyme kinetics in microgravity to better understand the mechanism action inside and outside biological cells. To do this DMLM III device was developed, which is responsible for mixing liquids in microgravity. It consists of a mechanical part, with ten sets of reaction systems, and embedded electronics. Each reaction system consists of three chambers, for the enzyme, the substrate and the reaction inhibitor, and two valves to keep the liquids separated. When microgravity signal is triggered, enzyme and substrate are mixed to start the biochemical reaction. After five minutes the reaction is interrupted with the inhibitor. This experiment was embedded on a VSB-30 Brazilian sounding rocket, and payload remained in microgravity for approximately six minutes. Biochemical analyses of the samples are under way, and the results will be compared with the data obtained on earth, under similar conditions.

1. INTRODUCTION

The effect of microgravity on some enzymes, responsible for pharmacological and immunological cells responses, has been investigated by different researchers, but the results are still contradictory [1].

Low gravity environment has proved to be favorable to the study of phenomena that are affected by gravity, such as fluids and heat behavior, and mass transfer. As a consequence of microgravity, hydrostatic pressure, potential energy and convection are minimized, and surface tension and viscosity become determinant components of the behavior of fluids in low gravity [1, 2]. Emulsions are more stable and basic phenomena, such as sedimentation, mixing, convection and diffusion, are considerably different in microgravity. Consequently, biological systems are also influenced by microgravity [3-5].

In the area of biotechnology there are many studies on biosynthesis of important pharmaceutical compounds, from animal cell cultures in bioreactors and cellular biodynamic in space environment. Works realized in growing protein crystals and cell cultures and tissues have been developed with great application potential for rational design of new drugs [3-6]. Research in microgravity could lead to a greater understanding of basic phenomena, and lead to benefits in applying this knowledge on earth and in the development of future applications in space.

The study of enzyme kinetics is essential to understand the mechanism of action of enzymes inside and outside cells, and for the design of enzymatic bioreactors. The influence of microgravity on the kinetic parameters of enzymatic reactions is poorly exploited. It is believed that significant differences may occur in the mechanism of action of enzymes due to the phenomenon of diffusion, which is altered in microgravity. Some works realized with plants showed that the enzymatic activity of several enzymes of the cell walls of plant cells increases in space, which causes a change in metabolism and cell wall structure [7]. The study of the effects of microgravity on the action of enzymes can produce knowledge to better comprehend, in the future, their mechanism of action on earth, and think of future applications of microorganisms and enzymatic processes in space.

In this experiment it will be adopted, as a model for studying the kinetics of enzymes in microgravity, the hydrolysis of sucrose by the action of invertase, present in cells of Saccharomyces Cerevisiae, because it is a well-known enzyme model on earth, which has been studied for some time by this research group. It is known that significant differences may occur in the mechanism of action of enzymes due to the phenomenon of diffusion, which is altered in microgravity. Some works realized with plants showed that the enzymatic activity of several enzymes of the cell walls of plant cells increases in space, which causes a change in metabolism and cell wall structure [7]. The study of the effects of microgravity on the action of enzymes can produce knowledge to better comprehend, in the future, their mechanism of action on earth, and think of future applications of microorganisms and enzymatic processes in space.

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The effect of microgravity on some enzymes, responsible for pharmacological and immunological cells responses, has been investigated by different researchers, but the results are still contradictory. The study of the microgravity effects on enzymatic catalysis includes, among the objectives, the comprehension of the cellular metabolism principles in microgravity and the use of enzymatic biosensors in space.

The goal of this experiment is to investigate the enzyme kinetics, in microgravity, which is essential to understand the mechanism of enzyme action inside and outside biological cells. This knowledge is fundamental to the study of cell metabolism (in vivo), for design of new drugs and for the bioreactors design for industrial enzyme (in vitro). Many cellular processes are modified when cells are exposed to microgravity conditions.

It is expected, as a result, the production of technoscientific knowledge in biotechnology, which will help to elucidate issues on reaction mechanisms, enzymes stability, and others.

2. PROJECT DESIGN REQUIREMENTS

In order to offer a wider range of samples to study invertase enzyme bioreactors performance, with different biochemical reaction possibilities, requirements were that at least ten different solution concentrations of invertase and sucrose should be sent aboard the sounding rocket payload, to be tested in microgravity. Previous experiments, with a single sample, had shown to be insufficient for study, because they did offer the possibility to confront a sample with its counterpart, submitted to identical reaction conditions.

On the other hand it was also important to duplicate samples, to ensure that, in case one system failed to work properly, there would always be another one to work with.

For these reasons, the initial project required that three identical sets of ten samples each, with ten different solution concentrations, would be used, both to ensure redundancy and untie divergence of data, in case of dispute.

However, for practical reasons, due to weight and volume available in the sounding rocket payload, the number of samples had to be reduced to five. Redundancy criterion was maintained, but not divergence clearing, because of the even number of sets reproduced.

3. BIOCHEMICAL PROCESS REQUIREMENTS

As it has already mention before, since the main objective of this investigation was to verify the behavior in microgravity of invertase on a sucrose substrate, in different concentrations, several tests were carried out in laboratory, in order to determine which the most convenient ones were.

In order to attend to the requirements of the main objective, chemical reactions should strictly happen while in microgravity: for this reason they should be started on entering microgravity and should be interrupted when on their leave.

To start the reactions it was only necessary to put in invertase and sucrose in contact at the right time.

On the other hand, to interrupt a reaction, once it has started it was necessary either to heat it up to a reasonably high temperature, over 90°C, or to introduce a reagent that did not interfere with the product of the current reaction.

The second alternative was considered to be more convenient and practical for this experiment, to avoid inserting a heater in the process.

Based on these requirements, three liquids should be used, that is invertase, sucrose as substrate and a reaction inhibitor. The three liquids should be sent separately in sealed chambers, so as to avoid any contact among them. In microgravity environment, invertase and sucrose should be put in contact to start reaction. On exiting microgravity environment, the reaction inhibitor should be added to the mixture in order to interrupt the ongoing reaction. Finally, the sample had to be recovered, after launching, for analyses.

4. DMLM III MINI-LAB

To perform the study of enzymatic reaction in microgravity, it was developed a new device called DMLM III. This device is responsible for mixing liquids in microgravity, and it consists of a mechanical part, with 10 sets of reaction chambers, and embedded electronics, powered by independent battery, that is responsible for the control of various functions. This was an evolution over the previous versions, which flew on VSB-30 sounding rockets in other Brazilian missions [11-15].

Each reaction system consists of three chambers and two valves that keep the liquids separated from each other. The mechanical part consists of two blocks, each one with five sets of valves and chambers.
interconnected, where the enzyme sample reacts with the substrate, at a certain concentration. These sets consist of three chambers and two valves. The enzyme is put in each chamber, as well the substrate and the reaction inhibitor. The chambers are separated by valves, ensuring the isolation of fluids.

When the microgravity signal is triggered, the first valve of each set is driven by a step motor system, which powers the entire valve assembly through a transmission lever. After this, the enzymes and the substrate, at different concentrations, are put in contact and the biochemical reaction starts. After 5 minutes, the second set of valves is driven by another step motor, which puts them in contact with the inhibitor to stop the biochemical reaction. During the experiment, the internal temperature is monitored. Data are periodically stored in the processor memory and also sent by telemetry.

5. MECHANICAL PARTS

DMLM III device was implemented to allow that biochemical reactions might happen, under control, while in flight aboard a VSB-30 sounding rocket, when entering microgravity environment. It is constituted of mechanical parts, controlled by embedded electronics.

Fig.1 shows some external and internal details DMLM III view, packed in its metal box. This package was developed specially for this experiment, meeting all requirements necessary for this application.

Fig. 2 shows internal details of DMLM III device. It is possible to notice the two reactions chambers set, arranged one over the other, the pack of batteries and the electronic boards.

Redundancy was adopted for safety reasons, so that two sets of five reaction chambers blocks were implemented, summing up a total of ten chambers. The original project had been designed for three chamber blocks, to ensure the validation of data by majority. The idea, however, was turned down, to attend to weight and volume available for scientific payloads on the sounding rocket, where the device would be embarked.

Each chamber block is made up of three steel chambers, as shown in fig. 3 and fig.4, which contain the three liquid reagents: the sucrose substrate, the invertase enzyme and the inhibitor reagent.

The three liquids are separated, in each chamber, before starting the experiment. When the sounding rocket trigger the microgravity signal, liquids A and B are put in contact by opening valve 1. After 5 minutes, valve 2 opens to allow the inhibitor liquid get in contact with the mixture, interrupting the chemical reaction. The
time was chosen so as to ensure that the process would only and completely be performed in microgravity environment, without any other external interference.

The five blocks of chambers are driven by a transmission shaft, to which they are connected, so that the valves may all open at the same time: this ensures that all chambers have exactly the same time slot reserved for reaction. There are two valves per block of chambers, in a total of ten, with two transmission shafts, each one controlling five of them: the first one puts the enzyme and sucrose in contact for reaction, and the second injects the inhibitor to stop the biochemical reaction. Step motor drive each shaft of each block set, to ensure simultaneity of events in all chambers.

Four step motors are used, two for each set of block chambers, as it can be seen in fig. 3, which are powered with 12 Volts, by a set of 10 batteries (1.2V each one).

The loading process of the liquids, each in its proper the chamber, was a particular problem to be dealt with, so as to avoid possible leakage points and to speed up load and reload time, before the rocket launching. As a matter of fact both invertase and sucrose needed to be replaced every 24 hours, because they lose their properties after this period time, if they are not kept in proper cooling conditions.

With this in mind, loading of liquids was devised to be performed with a syringe for each liquid, starting from the inhibitor, and then proceeding ahead with the other two. Each time a chamber is filled, the corresponding valve is closed, sealing off the liquid in that chamber and isolating it from the other chambers, which are cleaned up, to prevent any possible contamination in the process. The process goes on up to the last chamber in the row, which is sealed off with a screw.

The loading process of liquids, in all 30 chambers, after some training, takes about two hours to be completed. Steel chambers, as seen in fig. 4, were used not to react with the liquids of the experiment, which would adulterate the chemical reaction.

6. ELECTRONICS

DMLM III device is controlled by embedded electronics, which consist of a CPU-Central Processing Unit built around a microcontroller ADuC814 [16], to perform monitoring and data acquisition of variables for control, including chambers temperature, motor controls, data storage in flash memory, and serial communication for the rocket telemetry data system.

The microcontroller ADuC814 was chosen for its compactness, low power consumption, simplicity of use and multifunctional features, which reduced peripheral hardware. Since analog data had to be collected and stored, a built in 12-bit A/D (analog-digital) converter was a most convenient feature, and satisfactory for this application. It is an 8-bit microcontroller and it works with an external low clock frequency of 32 kHz, and internal 16.78 MHz [16]. The possibility of flash memory is also an important feature and an easy way for data recovery.

This microcontroller had already been used in other microgravity projects by our hardware designers, and had proved to be efficient and reliable [12-15].

Fig.5 shows some details of a DMLM III CPU used in the embedded electronics.

Redundancy was a constant in the project design, and it was implemented whenever possible: we also applied to the support of the sounding rocket telemetry system, to back up our data and, in the case of the rocket internal temperature, to confront it with our device. This would ensure that certain parameters, other than microgravity and other rocket mechanical interactions, might be reproduced on earth, in lab tests, to simulate the flight environment.

Power supply was provided with an independent set of 10 x 1.2V Nickel Metal Hydride batteries, to guarantee 12V at start up, with a 2,300mA current peak. This
particular type of battery was used for its stability and recharge facilities, with no memory effects associated.

A separate voltage regulator was used to ensure 5V supply to the electronic system and to the four motors, even when working in the worst conditions.

The step motor requires a current peak of 1,800mA at start up, and then current subsides, as it can be seen in fig. 6.

![Figure 6. Measurement of the electric current, when the first valve is set open.](image)

Separate power supplies, for the motors and the electronics, are provided, to avoid interference with each other.

The embedded electronics current does not exceed 140mA in steady state. In order to save energy, the CPU enables 12V power supply reserved to the motors only on entering microgravity, when the motor drivers are triggered. This ensures full operation energy to the system for the time that the experiment flies in microgravity.

Temperature data acquisition is performed by the microcontroller, through its A/D inputs, that read voltage levels of a temperature sensor transistor, and converts them in a 12-bit word. This word is then stored in memory as a 16-bit format, adding F to the four most significant bits. Fig.7 shows the temperature behavior, inside DMLM III, during the runtime in microgravity.

The temperature monitoring inside DMLM III showed that it varied within a comfortable range for the experiment along the flight in microgravity, from 27.5°C to 32°C, and this would be most important to reproduce the experiment in laboratory so as to compare the results.

To avoid unnecessary processing time, theses data are not processed in the microcontroller to scale them down to the suitable range, nor are they converted to decimal base.

![Figure 7. Temperature measured inside DMLM III device in microgravity.](image)

This feature is released to a software auxiliary application in C, which reads serial data, coming either from telemetry or any serial input RS232 like, or through a USB, and stores them in any notebook or PC memory. These data may also be shown on the screen of a notebook, in real time, so that it is possible to follow, through some parameters, what is going on in the DMLM III before and during the flight.

The word format for data serial transmission was simplified, and reduced to five one-byte fields, to specify identifier, status, CPU and data as follows:

<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$</td>
<td>Status (communication word)</td>
</tr>
<tr>
<td>Field 2</td>
<td>Status (Ground Status) or M (Microgravity Status)</td>
</tr>
<tr>
<td>Field 3</td>
<td>N is any hexadecimal digit corresponding to a CPU</td>
</tr>
<tr>
<td>Field 4</td>
<td>FX (MSB-Most Significant Byte, where F is hexadecimal, and X is any hexadecimal digit produced by A/D conversion)</td>
</tr>
<tr>
<td>Field 5</td>
<td>YZ (LSB-Least Significant Byte, where YZ are any two hexadecimal digits produced by A/D conversion)</td>
</tr>
</tbody>
</table>

Where:
- Field 1 = $ (word header).
- Field 2 = S (Ground Status) or M (Microgravity Status).
- Field 3 = N (N is any hexadecimal digit qualifier corresponding to a CPU).
- Field 4 = FX (MSB-Most Significant Byte, where F is hexadecimal, and X is any hexadecimal digit produced by A/D conversion).
- Field 5 = YZ (LSB-Least Significant Byte, where YZ are any two hexadecimal digits produced by A/D conversion).

Since A/D conversion is limited to 12-bit precision, the four most significant bits are set to F, simplifying mask and retrieval, besides consuming less energy.
7. DESIGN OF BLOCK CHAMBERS

As it was mentioned previously, a main concern in designing DMLM III device was, among others, proper sealing, both to protect other experiments, inside the rocket payload, from any sort of contamination or chemical damage, and to guarantee that the chemical reaction would be performed with the correct component specification, as to volume, mass and concentration. The results of the chemical reaction would therefore be analyzed and compared to similar reactions in laboratory tests, according to these parameters. This would lead to a specific design of block of chambers for the experiment.

The design of a block reaction chambers, serially connected and separated by a valve each, has proved to be effective to automate the mixture of liquid reagents in an established sequence. In this particular case the sequence was limited to two steps and three reagents. The system, however might work just as well for a larger number of steps, so that it could be generalized to work with \( n \) steps and \( n+1 \) reagents.

This architecture has also proved to be very convenient for loading liquids in chambers, to minimize leakage points, maintenance facilities, and processing time. The device was successfully submitted to all the required tests for flight validation. Another important aspect regarding block of chambers, is their serial and parallel organization. Serial organization guarantees the use of multiple reagents, to be activated automatically in a certain sequence and in due time.

Parallel organization, on the other hand, allow for simultaneity of actions. This was achieved with the use of a transmission shaft, which connects all the valves that correspond to a certain chamber in a row (serial). In this case it is possible not only to activate automatically different valves in a certain sequence, but also to establish \( "n" \) steps, for \( r \) reagents that must be activated simultaneously with the use of \( n \) motors.

8. CONCLUSIONS

The goal of this experiment was to investigate the enzyme kinetics in microgravity, to understand the mechanism of enzyme action inside and outside biological cells. To perform this study it was developed a new device called DMLM III, which is responsible for mixing liquids in microgravity. It consists of a mechanical part, with 10 sets of reaction systems, embedded electronics that is responsible for the control of various functions, and step motors to activate the valves of the reaction chambers.

This experiment was embedded on a VSB-30 Brazilian sounding rocket, which was launched on December, 12th, 2010 from CLA base, in Brazil, in cooperation with DLR - Deutschen Zentrums für Luft - und Raumfahrt. The mission was successful and the payload, which flew in microgravity for approximately 6 minutes, with 9 other Brazilian experiments, was rescued in high seas, eight minutes after its fall.

Biochemical analyses of the samples are under way, and the results will be compared with the data obtained on earth, under similar conditions. First results show an increase in enzyme activity in microgravity environment.

9. ACKNOWLEDGEMENTS

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10. REFERENCES

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