

AN INCREASE OF THE TOTAL SOLIDIFICATION TEMPERATURE IN SALT MEDIA BY DEXTRAN PROTECTION

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Summary

An investigation was performed on the possibilities for an alteration of the total solidification temperature when preserving concentrated salt media (BME, MEM, 199, HAM), used for the maintenance of animal viruses and cell cultures. It had been established that by the use of dextran 40000, this temperature could be increased with about 20°C. A protection in 3.6 % concentration was recommended and the freezing to minus 40°C might be performed directly in the refrigerator for storage. The temperature should not be raised over minus 32°C during the stockage.

Introduction

The maintenance of animal viruses and cell cultures is related to their multiplication in biological hosts and cryogenic conservation. It is accompanied by the application of synthetic media based on balanced salt solutions. These solutions, according to the investigations of Benet and Preaud [2], require special attention for the preservation of their qualities and homogeneity. The quantities of the media used in the collecting activity are comparatively small, both in the solutions (series) prepared in the labs and as industrial packing. On the other hand, the application of a controlled series nutrient medium is recommended for the achievement of standardisation in the work as long as possible. The decision can be

found in the preparation of concentrated media that divided into portions, to be stored as frozen. We have been using for years this way to standardise the conditions for cell cultures and viruses maintenance. It has to be mentioned that the total solidification temperature of the basic salt solutions is low. The ten-fold concentrated media completely freeze up at temperatures lower than minus 50°C - Table 1 [2]. This necessitates the application of low-temperature freezers providing minus 70°C for the conservation.

On the other side, the eutectic temperature of the quoted media is high and enables their storage in "low class" refrigerators providing temperature of minus 40°C.

Table 1. Temperatures of total solidification and eutectics, determined by G. Benet and J.M. Preaud according to the method for measuring of their resistance [2].

| Media | Total solidification temperature (°C) | Eutectic temperature (°C) |
|------------------------------------|---------------------------------------|---------------------------|
| Eagle (BME) [5] | minus 53 | minus 31.0 |
| Minimum essential medium (MEM) [6] | minus 52 | minus 28.5 |
| 199 in Earle's salts [8] | minus 64 | minus 30.0 |
| 199 in Hanks salts [8] | minus 52 | minus 29.9 |
| F10 of HAM [7] | minus 50 | minus 31.2 |
| Beef serum (natural) | minus 51 | minus 15.7 |

The low total solidification temperature for virus vaccines, obtained with these media, also brought problems to us during their lyophilization. The freezing time was prolonged, thence - the time for the vacuum sublimation drying. The production appeared to be more expensive. We realised, that by the cryoprotection, aiming at maximal preservation of the infectivity when lyophilizing viruses, the dextrans increased

the full solidification temperature of the suspensions [3]. This gave us a possibility to work out and introduce a new lyophilization technology for many vaccines [4].

Meeting again the problem for the cryopreservation of salt solutions and nutrient media for the purposes of virology, we set us the task of making detailed investigations on the possibility to increase their total solidification temperatures.

Materials and Methods

We provided the same apparatus, methodology and technical performance for to achieve comparability in the accomplished results with the ones of the scientists who had ever described such an investigation - G. Benet and J. M. Preaud [2].

Media. Five of the media were prepared and filtered through a membrane filter 0.22 µm:

- Basic media of Eagle (BME), without phenolic red, 10 x c [5];
- Minimum Essential Medium (MEM), with phenolic red, 10 x c [6];
- Medium 199 of Parker, with phenolic red in salts of Earle, 10 x c [8];
- Medium 199 of Parker, with phenolic red in salts of Hanks, 10 x c [8];
- F10 of HAM, 10 x c [7], beef serum from

forty-five-days-old and younger animals.

Dextran with a molecular weight of 40000 in concentrations from 2 % to 10 % was used as a protector.

Apparatus.

- Resistance measuring device "Tacussel" type CD 60, with limits from 0 to 20 MΩ, divided into seven ranges. The exactness of the evaluation was 0.01 MΩ.cm, with a platinum drill for estimating the resistance - GT 02/554;
- Temperature measuring and recording device, with a working range of 30°C to minus 180°C, provided with a drill RT 100;
- Ultracryostat for operation with liquid nitrogen "Nikol", providing constant freezing speed - 2°C.min⁻¹.

Excel was the program used for building up the graphic dependencies and deducing the results.

Working methods. The method of Rey [9] had been used. It was based on the resistance changes, depending on the temperature of the studied medium when freezing and melting. Six millilitres of the material were placed in a special glass chamber, provided with drills for estimating the resistance and the temperature. The sample was frozen down to minus 70°C at a speed of 2°C.min⁻¹ in an ultracryostat "Nikol". A styropor chamber with a wall thickness of 5 cm, providing a smooth increase of the temperature at a speed of 1 to 2°C.min⁻¹,

was used for the melting. The results achieved by five independent measurements were processed on a PC and graphics were build up. The temperature, at which a sharp rise in the resistance when freezing the sample had been registered - 15 to 20 MΩ.cm, was considered to be the total solidification one. The temperature at a resistance value of 0.01 MΩ.cm, achieved during the melting, was considered to be the eutectics[2].

We included observations about the appearance of side effects on the development of over 20 different cell cultures, cell lines and viruses, caused by dextran, as an additional investigation.

Results and Discussion

The addition of dextran to the different media obviously increased their total solidification temperature. The initial experiments made clear that the effective dextran concentration as a cryoprotector was 3 % to 4 %. Higher concentrations (up to 10 %) insignificantly increased the total solidification temperature and their application was unreasonable from an economic point of view. On the other side,

the physiological concentration of the dextran when used as a substitute in the blood plasma, had been 3.6 % [1]. On the basis of these data, we continued our investigations with the isotonic concentration of the dextran - 3.6 %.

The generalised results from the performed studies for the total solidification temperature of the investigated media determination, after the addition of dextran, are presented in Table 2.

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| F10 of HAM [7] | minus 30 | minus 31.0 |
| Beef serum (natural) | minus 31 | minus 15.5 |

When comparing the values with the ones in Table 1, we established that the total solidification temperature increased with more than 20°C for all the samples.

No change was registered in respect to

the eutectic temperatures.

The results from the biological experiments exhibited a complete indifference of the dextran in the nutrient media, towards the multiplied cell cultures and viruses (data not shown).

Conclusions

The accomplished investigations and the achieved results give us a reason to recommend a protection to be performed with dextran (40000) in a concentration of 3.6 %, when preserving concentrated media like BME, MEM, 199, HAM as frozen.

The cooling speed does not influence the process, so the freezing to minus 40°C may be performed directly in the refrigerator for storage.

The temperature is recommended not to be raised over minus 32°C during the stockage.

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