Storing Microdialysis samples

application note no 8

Introduction

One of the main characteristics of microdialysis sampling is the small volume involved. At a flow rate of 0.3 µL/min it is theoretically possible to collect only 18 µL during one hour.

Because of the small volumes, the samples are sensitive to evaporation even though they are "sealed " in the microvials. Furthermore, since the vials may contain different volumes, the effect of evaporation can vary between the samples. Thus, in order to get "quality" results, the samples should be analyzed as soon as possible after collection. Furthermore, chemical and microbiological degradation may also change the sample composition if the samples are stored for a long time.

Storage of samples

Samples can be stored in the refrigerator for a few days, but, in general, the colder the samples are kept, the lower the risk of chemical and microbiological degradation. However, when storing samples with small volumes evaporation must also be considered. Evaporation is less at lower temperatures but even if the samples are stored in a freezer, some evaporation may occur due to freeze-drying. To minimize this risk, it is important to keep the vials tightly sealed during storage. However, different materials contract differently upon lowering the temperature. Therefore in order to keep the rubber stoppers effective also at lower temperatures, the vials should be kept in Microvial Racks (Ref.P000028). Furthermore, the plastic used in the microvials is not gas tight and water vapor can escape through the plastic leading to freeze-drying of the samples. This effect can be minimized by storing the loaded microvial racks in sealed plastic bags while in the freezer.

Analyte stability at –20°C and –70°C

A small study was performed to monitor the stability of glucose and pyruvate in microdialysis samples collected from CNS and adipose tissue, at –20 °C and -70 °C for a period of about 3 months. The samples were stored in microvial racks that were kept in sealed plastic bags. At least four samples were analyzed at each test occasion and each storage temperature. The results are presented in the two figures below.

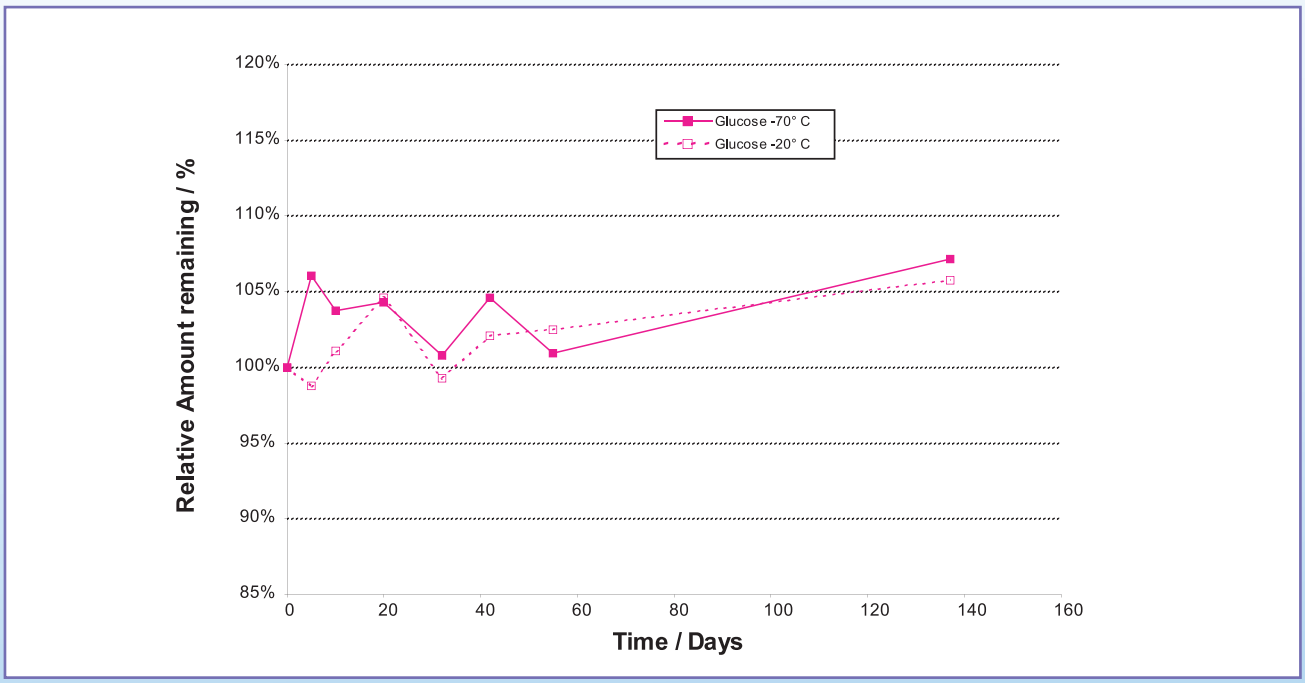

Fig 1. Average recovery of glucose after storage at –70°C and –20°C.

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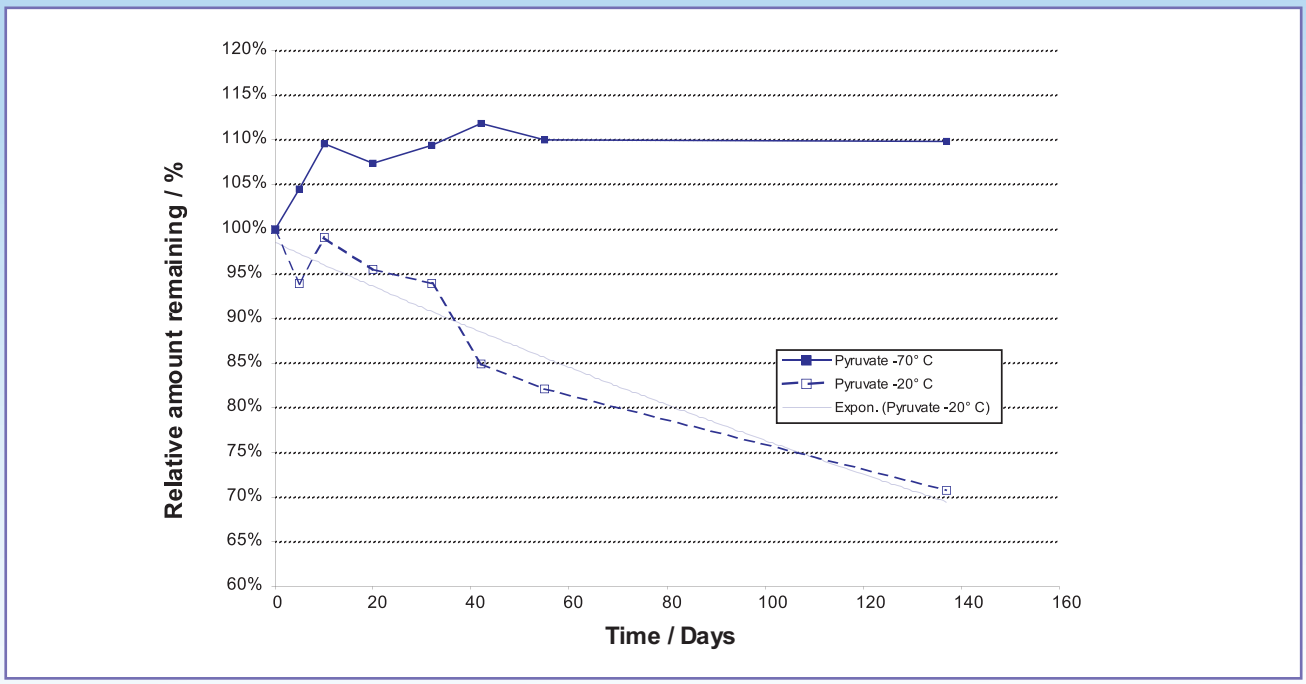

Fig 2. Average recovery of pyruvate after storage at –70°C and –20°C.

As can be seen in the figures, glucose is stable during the whole study period at both storage temperatures; the average recovery ± range was 102 ± 4% at –20 °C and 103 ± 4% at –70 °C.

When stored at –70 °C, pyruvate is also stable for three months; the average recovery ± range was 106 ± 6%. However, when stored at –20 °C a significant loss of pyruvate is found (about –10%per month). It is not recommended to store the samples for more than one month if the levels of pyruvate are important. No difference could be seen between the CNS and the adipose tissue samples. A slight increase in the levels for the samples can be noted during the course of the study, but it is not statistically significant. This increase is likely due to the freeze-drying effect discussed above. Furthermore, at the last test occasion, two of the´samples stored at –20 °C showed much lower pyruvate results than those obtained when the samples had been analyzed earlier. The most likely explanation is that the samples had been contaminated by some kind of microorganism during the course of the study. Since the normal microvials are not sterile, this can occur at any time when handling the samples.

Unfortunately no data for the stability of lactate, glycerol, glutamate and urea in microdialysis samples is presently available. However, there are no indications that these substances should present any stability problems in a similar time frame.

Thawing

If the samples have been frozen, then they must be thawed prior to analysis. It is important to be aware of things that may occur in the process of thawing that might cause problems during the analysis if not addressed.

Firstly, when the samples are melting the liquid phase will initially contain a very high concentration of salt and the analytes. As the thawing process progresses, this concentrated solution will slowly be diluted by the melting ice. Thus, there is a risk that the thawed samples are non-homogeneous. It is therefore recommended that the samples be thawed flat in the microvial rack, to reduce the diffusion distance, and then centrifuged prior to analysis.

Secondly, the solubility of gas in the liquid phase is higher at reduced temperatures (The solid phase does not contain any dissolved air). Thus, during thawing, the liquid phase can dissolve some air that can be released as small air bubbles in the samples as they reach room temperature. By decreasing the time the samples are at low temperature, less air will be dissolved. It is therefore recommended to thaw the samples as fast as possible, preferably in a heating cupboard at +40 C for about 10 minutes. Longer times and/or higher temperatures may result in a risk for unacceptable evaporation.

Finally, since the analyzer is using very small sample volumes, it is very important to have pressure equilibrium in the vials when the analyzer aspirates the sample. Normally this is accomplished by the design of the large rubber stopper in the wide part of the microvial that allows air to pass freely. However, during the thawing process, melted liquid can obstruct this air path, which may result in poor precision when the samples are analyzed. It is therefore recommended that the large rubber stopper be removed before the samples are assayed.

Batch analysis

Stored samples are usually assayed using the batch analysis cassette and it is convenient to fill all available positions with samples. However if the low volume samples sit for too long in the analyzer prior to analysis, the warm environment of the analyzer may result in unacceptable evaporation.