

Product Information

L-Glutamine Stability Study

L-Glutamine, unlike most other amino acids, is labile in solution, and that concerns may cell culture scientists. This essential amino acid is required by virtually all mammalian cells in culture. Although the metabolic destiny remains a matter of conjecture, we conducted studies designed to confirm glutamine's lability and the conditions under which its deterioration could be minimized.

The first series was designed to confirm the breakdown of L-glutamine as a function of time and temperature. Basal Medium Eagle (BME) with Earle's salts was prepared for cell culture use, i.e., dissolved in deionized water, supplemented with 2.2 g of sodium bicarbonate, and pH adjusted prior to sterile filtration. In addition, the medium contained 2 mM L-glutamine. Aliquots of the medium were stored at 4 °C, at room temperature (21 °C), and in an incubator at 35 °C. Samples were periodically harvested and derivatized with phenylisothiocyanate. Quantitative amino acid analysis was performed by reverse-phase HPLC and each sample analyzed in the presence of an internal standard. L-Glutamine peak heights were normalized and resulting data expressed as percent of glutamine remaining from the fresh BME preparation. Results of these studies are shown in **Figs. 1-3**.

Glutamine was found to be most stable in medium stored at 4 °C. Little or no breakdown was detected until the end of the first week and virtually no further change was observed during the remainder of the test

period. We recommend refrigeration storage for all media (**Fig. 1**), but do not recommend freezing liquid media because many components precipitate irreversibly when exposed to temperatures below 0 °C.

Breakdown occurred more rapidly at room temperature (**Fig. 2**), with approximately 20% of the glutamine deteriorating by day three. Deterioration occurred more rapidly when medium was stored at 35 °C (**Fig. 3**). Within nine days, glutamine degraded 50%, and less than 20% remained at the end of three weeks. These data underscore the need to replenish cell culture media every few days, and suggest that aliquots of media incubated to validate sterilization should be discarded.

The loss of bicarbonate and CO₂ with time from BME buffered with sodium bicarbonate, and the consequent rise in pH raised the question of glutamine stability. A second series of studies were conducted to assess the effect of pH on the stability of glutamine. Three 10 mM solution of L-glutamine in water were prepared at pH 3.0, pH 7.3, and pH 9.0, and stored at room temperature. Samples were periodically harvested and analyzed as described above.

Results of these studies are shown in **Figs. 4-6**. Glutamine was most stable at neutral pH, and deteriorated at acidic and basic pH at similar rates. Data suggest that at least some of the breakdown of L-glutamine observed in media at room temperature (**Fig. 2**) result from a rise in pH.

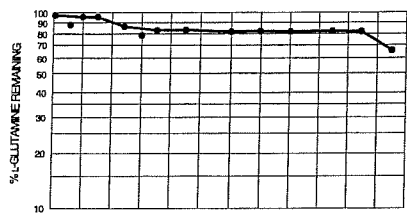


Fig. 1 DAYS IN STORAGE AT 4 °C

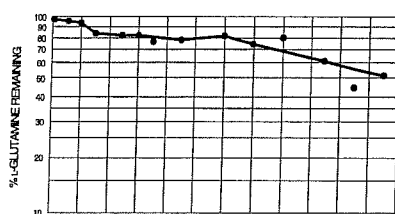


Fig. 2 DAYS IN STORAGE AT 21 °C

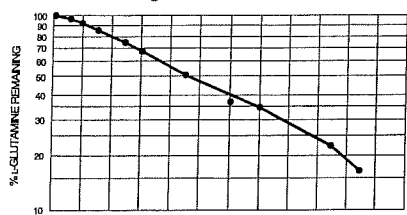


Fig. 3 DAYS IN STORAGE AT 35 °C

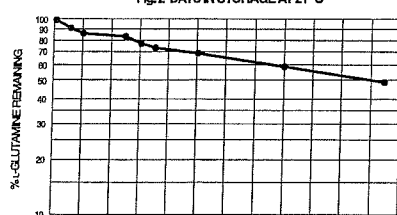


Fig. 4 DAYS IN STORAGE AT pH 3 AND 21 °C

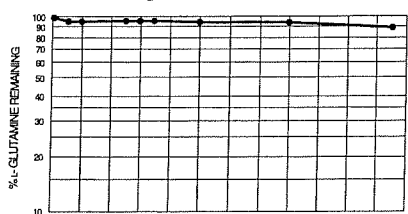


Fig. 5 DAYS IN STORAGE AT pH 7.3 AND 21 °C

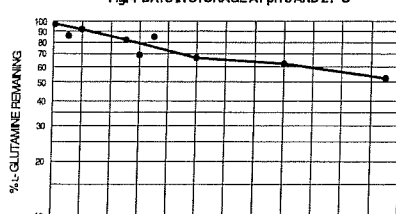


Fig. 6 DAYS IN STORAGE AT pH 9 AND 21 °C

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