Stability of diluted L-asparaginase in normal saline solution

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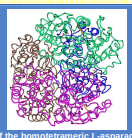
Background:

L-asparaginase (L-asp; Kidrolase®) is a homotetrameric enzyme used in the treatment of leukemia. However, its practical stability and optimal storage conditions have not been studied in detail. The aim of this study was to assess physical, chemical and biological stabilities of diluted L-asp stored during 10 days at 4°C.

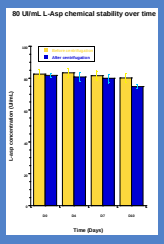
Materials and methods:

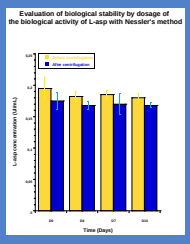
The following methods were used: size exclusion chromatography (SEC), dynamic light scattering (DLS) describing submicronic populations and corresponding mean diameters (md), turbidity (350 nm), thermal aggregation curves and determination of L-asp concentration by UV at 280 nm (chemical stability) and enzymatic activity (biological stability). Three batches were prepared under aseptic conditions in normal saline solution (80 UI/mL) in Freeflex® bags and stored at 4°C during 10 days. Aliquots were analysed at days D0, D1, D3, D4, D7, D9 and D10. Results were expressed as mean ± SD.



3D structure of the homotetrameric L-asparaginase enzyme with molecules of aspartic acid at catalytic sites.

$$\text{L-asparagine} + H_2O \xrightarrow{\text{L-asparaginase}} \text{Aspartic acid} + NH_3$$

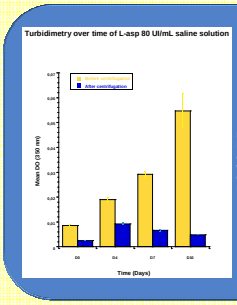




Chemical stability was assessed with the microdom® UV coupled to IR spectrophotometer. Absorbance was readed at 280 nm.

For biological activity study, each sample was diluted to 1/4000 just before analysis. Hence target biological activity at D0 was 0.02 UI/mL.

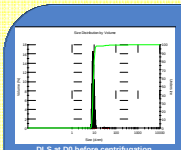
Centrifugation allowed us to see the formation of insoluble aggregates that still remained *active*.



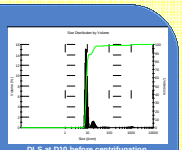
Formation of insoluble aggregates was exponentially increasing.

Results:

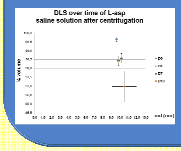
No significant difference was found both for chemical and biological activities after 1 week. The melting temperature was unchanged (59.0 ±0.1°C) (data not shown). Turbidity exponentially increased from 0.008 to almost 0.060 absorbance unit, indicating slight aggregation. Immediately after reconstitution, 4 peaks were found by SEC. The main peak (tetramer 133 kDa, 84% ±1% of the total area under curve) decreased to 70.4% ±7.9% after 10 days. These results were confirmed by DLS analysis since 3 initial submicronic populations were found: tetrameric population: md = 9.63 ±0.09 nm: 99.7% of the total population; highly aggregated populations: 50<md<200 nm; 0.3%. After 10 days, the md of main peak was unchanged but the percentage of tetramer decreased to 97.0% with an increase of the md of other populations (up to 900 nm). Percentages of aggregated enzyme (1.4%) remained unchanged during 8 days but reached to 9.6% at D10. However, the loss of enzymatic activity was only 6.1 % after day 10, suggesting that aggregated enzyme should partially retain asparaginase activity. In total, our results suggest that the loss of activity was not significantly modified until 8 days.



DLS at D0 before centrifugation

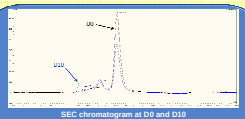


DLS at D10 before centrifugation

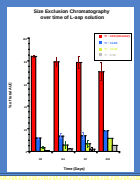


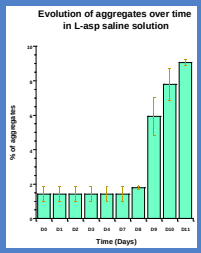
After 10 days, insoluble aggregates are formed and eliminated from the solution after centrigugation.

The main peak corresponding to the tetramer still have a mean diameter of about 9.6 nm.



SEC chromatogram at D0 and D10





Conclusion:

The results show that diluted L-asp in normal saline solution remains stable for 7 days at 4°C. Therefore, anticipated ready-to-use bags could be prepared by centralized pharmacy units and stored during 1 week without loss of activity. However, the slight increase of aggregates observed during the storage remains questionable in terms of potentially increased immunogenic-induced side-effects.