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## **Development of sustainable nanomaterials for the purification of antileukemic drugs**

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### **Highlights**

- Sustainable technologies to purify L-Asparaginase.
- Modified carbon nanomaterials have high affinity for L-Asparaginase.
- The pH and material/biopharmaceutical ratio influence the adsorption performance

### **1. Introduction**

Getting older is the biggest risk factor for most fatal diseases, including cancer, heart diseases and neurological diseases. To overcome such age-related diseases, it is crucial to optimize the production and purification of biopharmaceuticals, such as nucleic-acid-based products, antibodies and recombinant proteins and enzymes. Low cost production combined with high purity levels will allow their routinely use by a widespread population. Amongst the available biopharmaceuticals, continuous progresses have been carried out for the development of recombinant therapeutic enzymes, namely L-asparaginase (LA), which presents antineoplastic properties for the treatment of leukemia, namely Acute Lymphoblastic Leukemia (ALL) [1]. The first-line biologic used to treat ALL, Oncaspar, is currently marketed in the United States, Germany and Poland, accounting with approximately USD \$100 million in annual sales [2]. Given their high value, the demand for new biopharmaceuticals and cost-effective production/purification processes play now a priority role [3]. The common strategies for protein purification result in low yields and purity, requiring long processing times, while leading to a consequent increase of the process costs [4]. LA can be produced via fermentation and its purification usually comprises several steps. The methods to purify LA include precipitation, liquid-liquid extraction and chromatography techniques. Among these, ion exchange chromatography, which is often preceded by precipitation with salts as a first pre-chromatographic step, is the most used [4]. This work aims the development of cost-effective technologies to purify LA from the fermentation medium, which is rich in other proteins. Reusable functionalized nanomaterials, namely carbon nanomaterials (CNTs), are investigated as cost-effective purification techniques for the target enzyme, demonstrating to have high affinity for the target biopharmaceutical. The pH, material/biopharmaceutical ratio and contact time effects in the purity and yield of LA were optimized.



## 2. Methods

The surface chemistry of CNTs was modified by the treatment with nitric acid in liquid phase to introduce oxygen containing surface groups. Different CNTs were obtained and used for the purification of LA. Commercial LA was used for the first tests, in order to understand the behavior of the enzyme in contact with the nanomaterial. Experimental conditions, such as pH, and material/LA ratio, and contact time were studied. LA activity was quantified by Nessler reaction, which quantifies the amount of ammonium released after the enzymatic reaction [5].

## 3. Results and discussion

The first results reveal a total adsorption of LA by the CNTs. Depending on the CNT functionalization/ treatment, different values of recovered activity of LA were obtained. The pH and material/LA ratio also influence the adsorption of LA on the material surface.

## 4. Conclusions

The modified CNTs are promising nanomaterials for the purification of LA. The LA was easily attached to CNTs by adsorption under mild conditions. CNTs supports can be considered as a promising alternative for the adsorption and purification of LA.

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