**Ultrafiltration of Protein Based Solution. Study of Selectivity and Protein Conformation.**

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**Highlights**

* Membrane hydraulic and selectivity properties modified by lysozyme
* Lysozyme suffers changes in the hydrodynamic radius after filtration
* Denaturated form of lysozyme present after filtration

**1. Introduction**

With a growing market for them, proteins are intensively used in industry where they undergo different operations of separation, concentration and purification. A classical process used for such necessities is the membrane process.

Membrane processes use mild conditions of operation, have low energy consumption, no need for additives and can operate continuously. A key point to all the mentioned advantages is the possibility to modify the membrane properties to fit the need or demand of the industry. Thus, depending on the final outcome of the industry, membrane processes can give higher quality of the processed products. Nevertheless, membrane processes performances can be jeopardized by membrane fouling. Membrane fouling is a phenomenon in which, molecules from the liquid phase start to deposit or adsorb at the surface of the membrane or inside its pores. Fouling influences the membrane performances and in consequence, can affect the outcome of the process - economically and quality.

Our work consists in analyzing how proteins behave in membrane processes taking into account the evolution of the membrane performances and the transformation of the protein molecule.

**2. Methods**

Filtration of protein solutions was performed in a laboratory pilot-plant [1] using a tubular, ceramic asymmetric ultrafiltration membrane. In order to focus on the effect of the constrains generated by the change in pressure, temperature and flow rate are maintained constant.

The membrane performances after protein filtration were asset by determination of the selectivity (rejection rate) and of the hydraulic properties (permeability) using a neutral solution of Vitamin B12.

The possible change of the protein conformation after filtration was asset by High Performance Liquid Chromatography (HPLC) study, an analysis based on size-exclusion.

**3. Results and discussion**

A pre-established sequence of filtration was used in the current work as presented in Table 1. Between the lysozyme filtration tests series, neutral solution of Vitamin B12 followed by pure water filtration were performed to analyze the evolution of the membrane performances. It was found that the selectivity of the membrane increase with each filtration test while the permeability decreases. This behavior is specific to the phenomenon of adsorption. Thus, the lysozyme molecule could be adsorbed at the surface of the membrane or in the pores of the membrane. Further tests confirm this hypothesis.

Table 1. Sequence of filtration test with respective concentration, observed rejection rate and hydraulic permeability

|  |  |  |  |
| --- | --- | --- | --- |
| **Protein Solution** | **Concentration (mM)** | **R max**  **(%)** | **Lp**  **(10-14m3.m-2memb)** |
|  | | |  |
| VB12 | 9,22E-03 | 40 | 5.7 |
| Lysozyme I | 0,025 | 90 | 4.7 |
| Lysozyme II | 0,025 | 95 | 4.5 |
| Lysozyme III | 0.025 | 97 | 4.0 |
| VB12 | 9,22E-03 | 73 | 4.0 |

Analyses on the retentate (fraction of the feeding solution rejected by the membrane) and permeate (fraction of the feeding solution passing through the membrane) by HPLC confirm the change of the hydrodynamic radius of the molecule after passing through the membrane. Peak fitting performed on the HPLC chromatograms shows the different forms of lysozyme present in the permeate (native and denaturated) as opposed to the retentate, which shows only one form (native).

**4. Conclusions**

The current study gives a frame on the evolution of the membrane properties when using a protein solution, taking into account the protein behavior after filtration. The study confirms the adsorption of the lysozyme molecule in the pores, while making a further observation on the change of the protein molecule after filtration.

**References**

1. J. Bikai, L. Limousy, P. Dutournie, L. Josien, W.Blel, C. R. Chim. 18 (2015) 56-62.