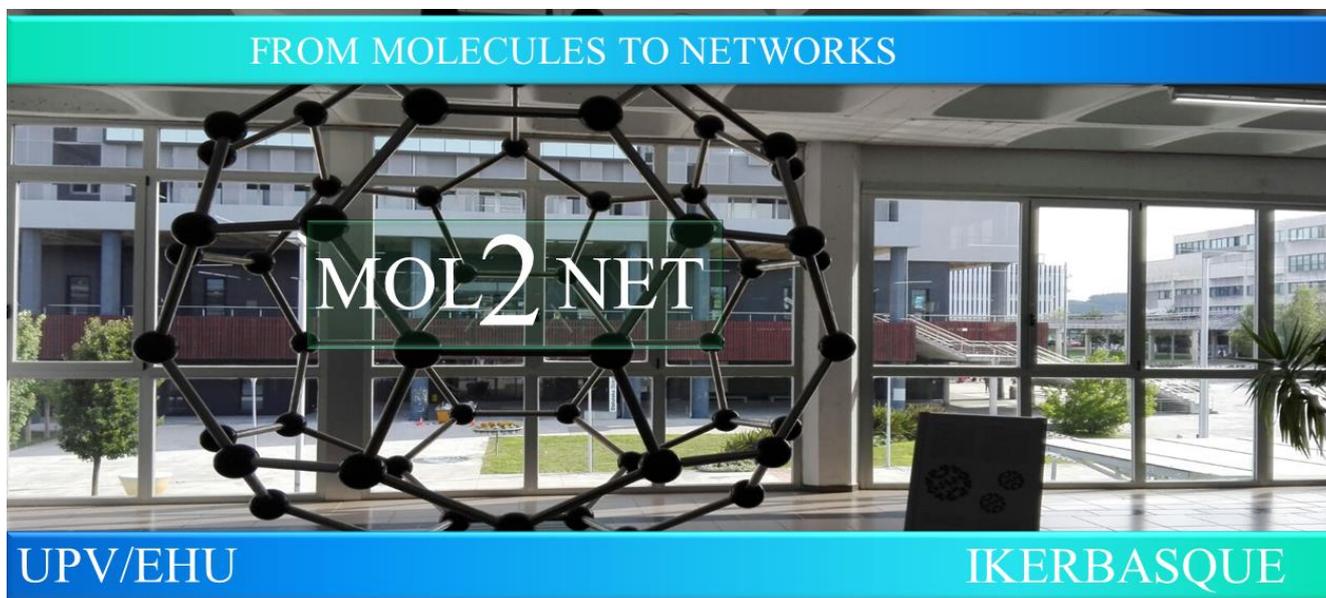




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Partial purification and characterization by fluorimetry and spectrophotometry of buffalo lactoferrin

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Introduction: Lactoferrin (Lf) is a iron ligating glycoprotein with a molecular mass of approximately 80 kDa, present in mammalian whey, belonging to the transferrin family. Lf can be found in various mucous secretions, such as tears, saliva, gastrointestinal fluids, urine and seminal fluid, as well as in secondary neutrophil granules, being released in places where there is an inflammatory response. Lf is a multifunctional protein possessing functions such as antibacterial, antiviral, antifungal, anti-inflammatory and immunomodulatory activities. This study aimed to purify, characterizing buffalo milk lactoferrin by monitoring the purification by spectroscopic techniques as well as investigating the interaction of protein with antibiotic amoxicillin. **Materials and Methods:** The processing of the buffalo milk began with the separation of fat by centrifugation. The skimmed milk was acidified with HCl 0.1 M up to pH 4.6, obtaining acidified whey and the sour serum was neutralized with NaOH 0.1 M until pH 6.8 and then centrifuged. The supernatant was submitted to saline precipitation profiles of 0-20%, 20-40%, 40-60% and 60-80% saturation of $(\text{NH}_4)_2\text{SO}_4$. Fluorimetric analyses of salt fractions were performed under excitation length conditions at 290 nm and emission wavelengths between 300-550 nm.

Spectrophotometric studies were carried out with additions of 100 μL of saline fraction 40-60% (0.421 mg/mL), 100 μL of purified lactoferrin (0.421 mg/mL) and 100 μL amoxicillin (2.5×10^{-6} mol. L⁻¹). UV-vis absorption spectra were recorded from 190 to 450 nm. **Results and Discussion:** The saline profile of the precipitate was resuspended 40-60% showed the spectrum of fluorescence extinction characteristic of lactoferrin (peak at 332 nm). The 40-60% precipitate was resuspended and submitted to liquid chromatography in a Sephacryl S-100 gel column. Fractions 12 to 16 showed the fluorescence extinction spectrum characteristic of lactoferrin. SDS-PAGE 8%, using commercial lactoferrin (SIGMA) as standard, showed the presence of two protein bands in the standard range. The UV-vis spectrum of maximum absorption showed that the interaction between lactoferrin and amoxicillin play an important role, with the decrease and displacement to the red peak on the UV-vis absorption spectrum when compared with that of lactoferrin partially purified with lactoferrin in the presence of the amoxicillin. **Conclusions:** Buffalo lactoferrin was partially purify by liquid chromatography in a Sephacryl s-100 resin and SDS-PAGE 8%, using commercial lactoferrin (SIGMA) as standard, showed two protein bands in the standard range. Partially purified buffalo lactoferrin exhibited a UV-vis absorption spectrum with two peaks; the first strong peak centered at absorption maximum in the region 220 to 230 nm is characteristic of the peptide structure and the second peak absorption maximum in the region from 270 to 280 nm due to conjugate double bonds in tyrosine and tryptophan residues. On assays of partial purified Lf incubated with amoxicillin was observed observe a redshift shift from 222.50 nm to 225 nm followed by the hypochromic effect on the UV-vis spectrum of maximum absorption of lactoferrin. The UV-visible absorption spectra studies showed that amoxicillin when coupled with lactoferrin induced alterations in the protein structure.

Keywords: Lactoferrin, purification, Fluorimetry, Spectrophotometry.