

## Synthesis, preparation and characterization of novel nanostructures of deferasirox-lactoferrin conjugates for metal chelation therapy in neurodegenerative diseases

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### Introduction:

Many neurodegenerative disorders including Parkinson's disease and Alzheimer's disease have been found to be associated with increased brain iron levels. Evidence shows that iron may damage brain tissues either directly or by changing the cellular environment and chelation therapy is considered to be one of the important approaches in the management of these disorders [1].

Lactoferrin is among the protein which is used for brain drug delivery as a vector. Lactoferrin receptors are greatly upregulated in the brain of patients suffering from neurodegenerative disorders which makes this protein a promising vector for drug delivery to brain in these diseases [2]. Here in, the conjugation of an iron chelator agent, deferasirox, with lactoferrin have been used for preparation of water soluble conjugates which may have the potential for brain drug delivery in neurodegenerative diseases.

### Methods:

Lactoferrin was used for deferasirox conjugation reaction. Briefly, deferasirox (18 micromoles) was dissolved in 1ml dimethyl sulfoxide (DMSO) and the mixture was added to 1 ml of a 70:30 mixture of DMSO:dimethyl formamide (DMF) containing 36 micromoles of EDC and 36 micromoles of *N*-Hydroxysulfosuccinimide (SNHS). The reaction was kept in room temperature for 5 hours under constant stirring. The resulting activated deferasirox was used to derivatize lactoferrin. A 50 microM lactoferrin solution was used to react with suitable molar excess of activated deferasirox of 20 times. This reaction mixture was kept overnight in room temperature under constant stirring. The reaction mixture was then centrifuged at 5000 g for 15 minutes in the room temperature and supernatant was concentrated and purified by Amicon® ultra-15 centrifugal filter devices and extensive dialysis.

Degree of conjugation was calculated as deferasirox number of moles attached to 1 mole of lactoferrin. Deferasirox was quantified by a UV spectrophotometry method according to one of the drug's properties. Deferasirox chelates iron in the presences of low pH and these colorful complexes were used for determination of deferasirox loading. An accurate and precise method for determination of deferasirox was established accordingly. In this method deferasirox UV absorbencies were measured after chelation with FeSO<sub>4</sub> solution in acidic pH in 515 nm and the protein concentration was determined by a modified Bradford method [3]. In addition the conjugated Lf was further characterized by gel permeation chromatography (GPC) on a TSK G2000SW column (7.5mm ID, 600mm L, particle size 10 µm, pore size 125Å) on an HPLC system (Agilent 1100 liquid chromatographer, Agilent technologies, USA) at 280 nm. A mobile phase of 0.2 M potassium phosphate buffer with a 0.8 ml/min flow rate was used. The size and size distribution of the resulted conjugates were analyzed by Zetasizer Nano ZS (Malvern Instruments, UK).

### Results:

The scheme of Lf reaction with deferasirox is shown in figure 1. As it shown EDC has been used as a water soluble carbodiimide coupling reagent to form an amide bond between the lysine amines of the protein and the carboxylic groups of deferasirox. For the determination of conjugation ratio a calibration curve was prepared with appropriate solutions of deferasirox which showed a good correlation ( $R^2 = 0.99$ ) in the calibration range and the method was shown to be precise and accurate (Figure 2). The conjugation ratio was determined to be  $6.544 \pm 0.602$ . The conjugated proteins were analyzed by GPC and an increase in the size of conjugated proteins was observed (Figure 3).

The conjugates had an average size of 106 nm and a particle size distribution was reported to be 0.345. Most of the particles had an average size of 79.1nm.

### Conclusion:

Water soluble nano structured conjugates of deferasirox were prepared by carbodiimide coupling mechanism and the conjugation ratio was determined. An increase in the size of the conjugated proteins

was evaluated and confirmed by GPC. The current conjugates have the potential to be used in neurodegenerative diseases to target brain tissues which suffer from iron deposition.

**References:**

- [1] Ana L, Sato H, Konishi Y, Walker D. G., Beache T. G. Rogerse J, Tooyamaa I; Neuroscience Letters **452** (2009): 277–280
- [2] Schneider S A, Hardy J, Bhatia K P; J Neurol Neurosurg Psychiatry **80** (2009): 589-590
- [3] Zor T, Selinger Z; Theoretical and Experimental Studies Analytical Biochemistry, **2** (1996): 302-308.

**Figures:**

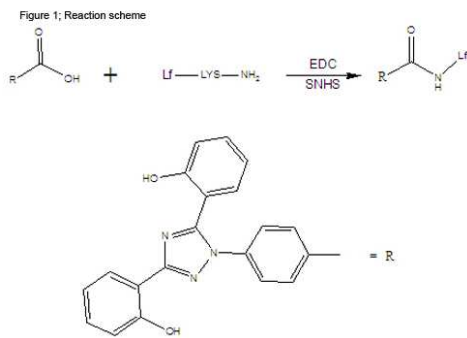


Figure 1: The conjugation reaction scheme between Lactoferrin and deferasirox

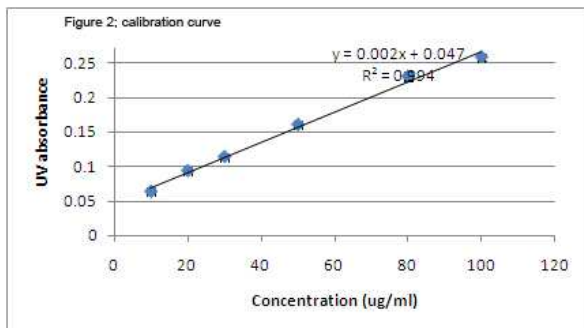


Figure 2: Deferasirox quantification calibration curve by UV spectrophotometry in 515nm

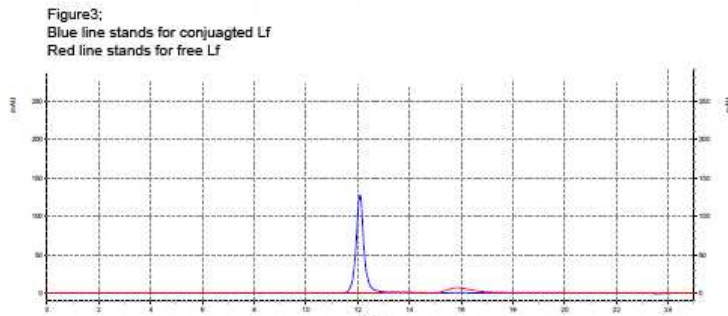


Figure 3: GPC chromatograms of conjugated lactoferrin (blue line) and free lactoferrin (red line)