ABSTRACT

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Title of Thesis: Spectrophotometric assessment of iron chelation at different pH

Iron is an essential element for living cells. Dangerous for the organism can be its lack and abundance as well. Therefore, its level must be meticulously regulated. The most common cause of iron overload is the excess supply. The iron overload is mainly brought about frequent use of transfusion or hematologic diseases. Iron toxicity is based on the ability to catalyze the formation of free radicals that modify cellular macromolecules and cause tissue damage. The use of iron chelators is therefore a rational therapy of iron overload conditions.

Iron chelators are molecules with different structures sparing the ability of iron binding, which results in iron elimination from the organism and prevent iron accumulation in tissues. It is mainly used for intoxication, but some of them are also used in other indications. Attention is paid to the chemotherapy and the treatment of neurodegenerative diseases.

There are many methods for determination of iron levels. Ferrozine method can be successfully used for the assessment of ferrous ions chelation. But this methodology is not applicable for the direct determination of the chelation of ferric iron ions. That can be provided by direct spectrophotometry. Ferrozine method cannot also accurately determine the complex ratio of the chelating compound:iron.

The aim of this work was to create and standardize screening methodology for the determination of the ability of different compounds to chelate iron by spectrophotometrical approach. At the same time in case of positivity, i.e. the ability of a tested compound to chelate iron, should allow the determination of stoichiometric ratio of the formed complex.

They have developed new modified *in vitro* methods to detect iron-chelating properties at different pH (4.5, 5.5, 6.8 and 7.5) by direct UV-VIS spectrophotometry. Active compounds form complexes with ferrous/ferric ions, which have different absorption maximum, than the original compound. The simplest measurement is the recording of absorbance at absorption maximum of the complex. This methodology is useful only in limited cases where the difference between the absorption maximum of pure substances and its complex is large. However in most cases (the difference between the absorption maximum of pure substances and its complex with iron is small), this requires more sophisticated methods based on the evaluation of the molar absorption coefficient of the test substance and its complex.

Stoichiometric ratios measured for known chelators (deferiprone, 8-hydroxyquinoline) is consistent with the available literature sources and demonstrate the correctness of the methodology used. In the case of flavonoids, available studies differ published stoichiometric ratios. Therefore, this method is a promising approach for the determination of iron-chelating activity of flavonoids in particular. Quercetin was chosen as a model compound. It was shown that quercetin with the exception of pH 4.5, when quercetin didnot chelate iron ions, formed complexes with 1:1 stoichiometry in all other experimental conditions.