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A comparison of photometric methods for serum iron determination under flow analysis conditions

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ABSTRACT

In this contribution, a multicommutated flow analysis (MCFA) system for serum iron determination is presented. The construction of the system has enabled sequential measurements with three different determination methods with 1,10-phenantroline, Ferrozine and Ferene S using separate analytical units (modules). For each method a dedicated optoelectronic detector has been developed. Under optimal flow conditions, investigated methods were analytically characterized by linear range and sensitivity: 1.5 – 100.0 μmol L^{-1} and 4.9 mV L μmol^{-1}, 3.3 – 50.0 μmol L^{-1} and 6.6 mV L μmol^{-1}, 4.0 – 40.0 μmol L^{-1} and 13.3 mV L μmol^{-1} for 1,10-phenantroline, ferrozine and ferene S methods, respectively. In the course of investigations also clinical usefulness of each method has been discussed. The analysis of serum samples proved that Ferrozine and Ferene S methods are sufficient for serum iron determination with relatively low consumption of samples (less than 50 μL of serum).

1. Introduction

Without any doubt iron is one of the most crucial trace elements in the body. Iron takes place in various metabolic pathways because of its ability to participate in reduction-oxidation reactions, both as electron donor and acceptor [1]. Iron as a cofactor of many iron-containing enzymes is responsible for energy metabolism, oxygen transport as well as DNA synthesis and repair [2]. Unfortunately, these features also determine toxic properties of iron ions. Ferrous ions (Fe^{2+}) cause the formation of hydroxyl radicals in the course of Haber-Weiss-Fenton sequence of reactions [3], contributing to lipid peroxidation [4], DNA damage [5] and neurodegenerative diseases [6,7]. As a result, iron practically does not exist in free form as ferrous or ferric (Fe^{3+}) ion and is rigorously controlled by homeostatic control system composed of many different regulatory proteins [1–3,5]. One of the most important clinical iron parameter is serum iron which refers specifically to the ferric ions reversibly bound to transferrin [8]. The reference range for total serum iron is between 10 and 30 μmol L^{-1} [9]. Every other value of serum iron level may indicate iron metabolism disorders which can be define as iron overload or iron deficiency, a single-gen disorder leading to abnormally high iron absorption [10,11]. The first pathological state is most frequently associated with hereditary hemochromatosis. In turn, the second one consists in reduction of iron stores usually by anemia. The problem of anemia due to iron deficiency is global and refers to 15% of whole human population, occurring children, teenagers and women in reproductive age, especially pregnant women [12,13].

The iron determination methods under flow conditions are widely described in the literature. In the last decade, many varied flow analysis systems have been proposed, e.g: flow injection analysis systems with photometric [14,15] and chemiluminescence detection [16], stopped-in-loop flow analysis with iron-catalyzed oxidative reaction of *p*-anisidine [17], sequential injection analysis with spectrophotometric reagent – deferiprone [18] or novel proposition in the form of cross injection analysis system using 1,10-phenantroline [19]. Thus far, majority of published papers refers to synthetic and water sample analysis and only a few articles about flow analysis systems for serum iron determination using photometric detection were presented [20,21]. Surprisingly, none of them shows the concept of Multicommutated Flow Analysis (MCFA) methodology [22–25], which can be really effective in the case of clinical analysis [25,26].

In presented contribution, a MCFA system based on dedicated flow-through optoelectronic detectors made of paired light emitting diodes [27–31] is proposed for analytical comparison of three photometric methods for iron determination. The aim of such study is to characterize iron determination methodologies in human serum samples under flow analysis conditions. In the course of investigations, a fully-mechanized MCFA system with solenoid

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microdevices has been designed, programmed and optimized. The selection of optimal measurement conditions was crucial for the comparison of proposed serum iron determination methods. The analytical and clinical usefulness of presented system was confirmed by human sera analysis.

2. Experimental

In the course of investigations three iron reagents for photometric determination of serum iron were used: 1,10-phenantroline [8], Ferrozine [32,33] and Ferene S [32,34]. Ferrozine (82950, ≥97%) and Ferene S (82940, ≥99%) were obtained from Sigma-Aldrich (USA). 1,10-phenantroline (412950224, ≥99%) was purchased from Avantor Performance Materials (Poland). As a reducing agent of ferric ions to ferrous ions, ascorbic acid (529150113, ≥99%) from Avantor Performance Materials was used. Iron (III) chloride hexahydrate (31232, ≥99%) for standards preparation, bovine serum albumin BSA (A7906, ≥99%) and reagents to reduce interferences: thiourea (T7875, ≥99%) and guanidine hydrochloride (G3272, ≥99%) were purchased from Sigma-Aldrich (USA). Other reagents of analytical grade, were obtained from Avantor Performance Materials (Poland). For all experiments doubly distilled water was used throughout. The control sera were purchased from Sero AS (Norway) and Cormay (Poland). The human serum samples with known concentration of serum iron were obtained from the Central Clinical Laboratory of Medical University of Warsaw. The reference serum analyses were performed with clinical analyzer Cobas Integra 6000 (Roche Diagnostics, Switzerland). For obtaining preliminary results the Shimadzu spectrophotometer (model PC 2401, Japan) was applied. These measurements were performed in disposable acrylic cuvettes from Sarstedt (no. 67.755, Germany).

For photometric determination of iron in MCFA system two kinds of optoelectronic detectors, operating according to paired-emitter-detector diode (PEDD) principle [28,29], have been developed. PEDD detector adapted for 1,10-phenantroline method was constructed by pairing of LED-emitter of 525 nm 50Cd (OSPG5111P) with LED-detector of 525 nm 10 Cd (OSPG5131A-ST). In the case of Ferrozine and Ferene S reactions, the chosen diodes were: LED diode 570 nm 1.5 Cd (LL-503UGG-2BC) as light emitter and LED 600 nm 4.2 Cd (OSYL5161A-NO) as light detector. All LEDs were purchased from Optosupply (Hong Kong). The architecture of flow-through detection cell was similar to the one presented in earlier publication [35], but this time the dimensions of 20 mm optical path and diameter of 3 mm were chosen. The internal volume of such optical cell is ca. 140 μL. The integrated flow-through PEDD detectors were prepared using micromechanical methods of fabrication in polyether ether ketone (PEEK) polymeric block.

PEDD detectors, were powered with stable current using Arduino Mega 2560 (Arduino, Italy) [36]. The analytical signal (electromotive force) was measured and recorded using UT70 B multimeters (UNI-T, China) connected with data storage computer via RS232 interface. The developed MCFA system consisted of solenoid micropumps (indicated stroke volume of 10 μl, product no. 120*SP*1210-4TE) and three-way solenoid microvalves (product no. 100T3MP12-62-5) which were purchased from Bio-Chem Fluidics (USA). These devices were powered and controlled using the above-mentioned Arduino Mega 2560 microcontroller board based on the ATmega2560. The scheme of connection between Arduino and solenoid devices through linear integrated circuit ULN 2803 as well as the program controlling whole system written in Arduino C language, which is the set of C/C++ functions [37,38], are given in the supplementary material. All flow system connections were arranged using PTFE Microbore tubing (ID 0.8 mm) from Cole-Palmer (USA).

3. Results and discussion

3.1. Optoelectronic detector

The first step in constructing of flow analysis system was to develop PEDD detectors for further experiments. For this purpose, absorption spectra of ferrous ions complexes with studied reagents (1,10-phenantroline, Ferrozine, Ferene S) were performed. The obtained spectra were compared with spectra of light emitting diodes which are the part of PEDD detection system. The process of LED emitters and detectors selection for PEDD construction was similar to the procedure presented elsewhere [39]. In Fig. 1, results for specified iron reagent and corresponding pairs of diodes are shown. For measurements with 1,10-phenantroline 525 nm 50 Cd diode as LED-emitter and 525 nm 10 Cd diode as LED-detector were chosen as a pair providing the best analytical parameters. In the case of two other iron reagents (Ferrozine and Ferene), 570 nm 1.5 Cd LED as emitter and 600 nm 4.2 Cd LED as detector were applied.

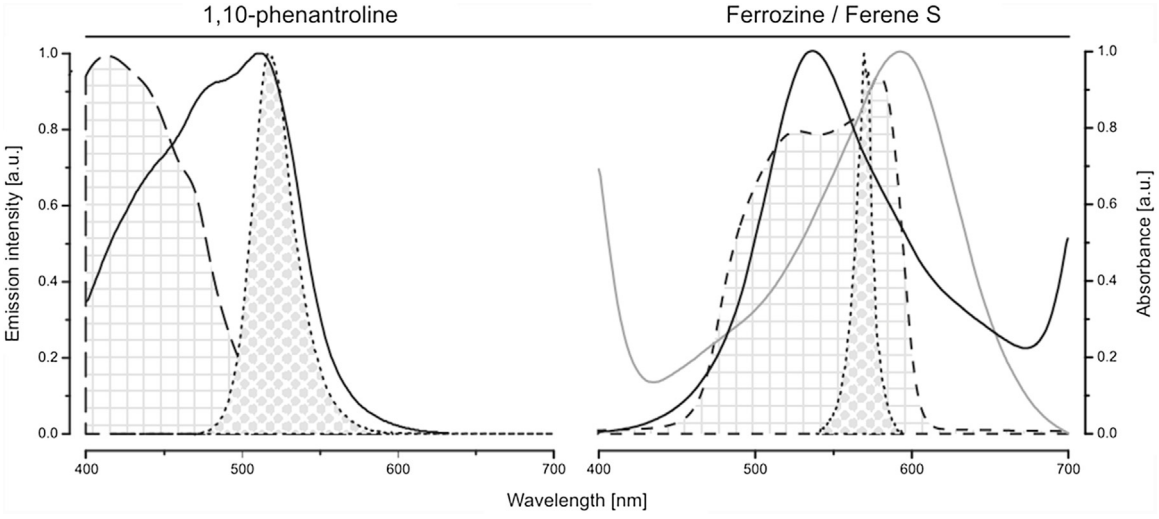

Fig. 1. The normalized absorption spectra of ferrous ions with chosen reagents and imposed emission spectra of LED-emitters and spectral sensitivity of LED-detectors. A) spectra for 1,10-phenantroline (no fill), 525 nm LED-emitter (dotted fill) and 525 nm LED-detector (lined fill); B) spectra for Ferrozine (black, no fill), Ferene S (grey, no fill), 570 nm LED-emitter (dotted fill) and 600 nm LED-detector (lined fill).