

Validation of analytical method for determination of iron ions on cotton materials

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Metal ion that remain on cotton materials during the production and finishing process of textiles cause damage. This is particularly pronounced if textile comes into contact with oxidising agents, such as hydrogen peroxide. The residual iron ions lead to the catalytic decomposition of H_2O_2 and the formation of hydroxyl radicals, which cause the cellulose to break down. The textile material loses its strength and dimensional stability resulting in degradation. To minimize the undesirable effects, it is necessary to monitor the concentration of iron ions on the textile material. The quantification of iron ions can be carried out using various instrumental techniques (UV/Vis spectrophotometry, ICP-OES, ICP-MS, AAS, GF-AAS, etc.). In this research, the advantage was given to UV/Vis spectrophotometry as a fast, simple and economically viable method. The content of iron ions on textiles after wet digestion was monitored spectrophotometrically using 3-(2-pyridyl)-5,6-bis-(phenylsulfonic acid)1,2,4-triazine disodium salt (Spectroquant®) as a specific reagent whose complex absorbs at 565 nm. The selected method was developed as an in-house method. Therefore, the validation was performed by determining the following performance characteristics: linearity, working range, sensitivity, selectivity, precision, accuracy, limit of quantification and detection, robustness and the cost of the method was estimated.

Keywords: iron; cotton; wet digestion; UV/Vis spectrophotometry; method development; validation

Izvorni znanstveni rad

Validacija analitičke metode za određivanje iona željeza na pamučnim materijalima

Ioni metala zaostali na pamučnim materijalima tijekom procesa proizvodnje i oplemenjivanja tekstilija uzrokuju oštećenja istih. Oštećenja su posebno izražena kada tekstilija dođe u kontakt s oksidacijskim sredstvima, poput vodikova peroksida. U prisutnosti iona željeza dolazi do katalitičkog raspada H_2O_2 i stvaranja hidroksilnih radikala, što rezultira cijepanjem celuloze. Tekstilni materijal gubi čvrstoću i dimenzijsku stabilnost te dolazi do njegove razgradnje. U svrhu minimiziranja neželjenih efekata potrebno je pratiti koncentraciju iona željeza na tekstilnom materijalu. Kvantifikacija iona željeza može se provesti različitim instrumentnim tehnikama (UV/Vis spektrofotometrija, ICP-OES, ICP-MS, AAS, GF-AAS, itd.). U ovome radu prednost će se dati UV/Vis spektrofotometriji kao brzom, jednostavnoj i ekonomski isplativoj metodi. Sadržaj prisutnih iona željeza na tekstilijama nakon mokrog spaljivanja praćen je spektrofotometrijski uz 3-(2-piridil)-5,6-bis-(fenilsulfonska kiselina)1,2,4-triazin dinatrijevu sol (Spectroquant®) kao specifičan reagens, čiji kompleks apsorbira pri 565 nm. Odabrana metoda razvijena je kao vlastita metoda, pa je provedena validacija određivanjem sljedećih izvedbenih značajki: linearnost, radno područje, osjetljivost, selektivnost, preciznost, istinitost, granica kvantifikacije i detekcije, robusnost te su procijenjeni troškovi metode.

Cljučne riječi: željezo; pamuk; mokro spaljivanje; UV/Vis spektrofotometrija; razvoj metode; validacija

1. Introduction

Textile product often contain organic and inorganic substances including metal ions [1], which can be originally found in natural fibers. Alternatively, metal ions can penetrate into textiles through used improperly prepared water during the finishing process, technical grade chemicals, or process equipment. For example, in the dyeing process various metal complex dyes are used [2]. Also, metals are added to improve wet fastness [3], or used as catalysts in the synthesis of dyes [4], so technical grade dyes will carry residual amounts of metals. Metal ions can remain after cross-linking (Sb, Sn, Zn, Cu) or condensation of resins (Zn and Al) [5]. In addition, they are used in finishing processes to enhance the properties of finished textile product. For example, for weighting silk [6], achieving water-repellent, antimicrobial [7], and flame retardant properties [8]. Usual iron conc. in waters are around $0,5 \text{ mg dm}^{-3}$, while underground waters can contain up to 10 mg dm^{-3} of iron. In a dissolved oxygen absence and at the usual pH for groundwater (pH 5-8), iron is dissolved in the form of ferric compounds (Fe^{2+}). In O_2 presence (surface waters) iron is oxidised forming insoluble iron hydroxide $\text{Fe}(\text{OH})_3$, which contributes to increased water turbidity [9-11].

It is assumed that metal ions bind to textile materials mainly through ion exchange on free hydroxyl and carboxyl groups of cellulose, which are formed by oxidation of hydroxyl groups during chemical processing. Even low concentration of metal ions can cause local fiber damage, which ultimately affects the quality of the finished product. Negative effect on textile material occur at precisely defined and characteristic concentrations for each individual metal ion. In bleaching process of textile materials, iron and copper ions at concentrations greater than 10 mg dm^{-3} catalyze the uncontrolled decomposition of hydrogen peroxide with the formation of highly reactive radical species ($\text{HO}\cdot$, $\text{HO}_2\cdot$), oxidizing the cellulose molecule with the formation of a series of products known as oxycellulose. The cellulose oxidation on C1 and C4 position directly causes the breakage of the cellulose macromolecule resulting in a decrease in the degree of polymerization (DP) and with that associated decrease in mechanical properties. An increase in the number of free aldehyde groups ($\text{R}-\text{CHO}$) enhances the reducing properties of cellulose. If materials exposed to latent oxidative damage on C2-OH and C3-OH are subjected to the washing process in alkaline baths, hydrolysis occurs. Ultimately, COOH carboxyl groups are formed and the cellulose macromolecular chain is depolymerised [12]. Taking into account the solubility product

constant $\text{Fe}(\text{OH})_3$ ($K_{\text{SP}} = 4,5 \cdot 10^{-37} \text{ mol}^4 \text{ dm}^{-12}$) at pH 3.13 iron is already present in the form of hydroxide $\text{Fe}(\text{OH})_3$ [9]. In alkaline baths, the iron in a form of precipitated hydroxide and fine metal filings from the air, is adsorbed onto the textile material. Under these conditions, iron hydrolyses with the release of protons, creating locally very acidic areas that promote the hydrolytic degradation of the cellulose. Moreover, iron present at concentration greater than $0,2 \text{ mg dm}^{-3}$ causes uneven coloring, quenches the fluorescence of optical brighteners, reduces the whiteness of textile materials, and reduces the effect of UV protective properties [13]. The above facts raise the imperative of continuous monitoring of iron ion concentrations in wastewater, but also on textile materials. It should be noted that continues monitoring of iron content must be integral part of every technological process.

Validation is a process of determining whether an analytical method is suitable for its intended purpose and is performed after method development. It ensures the credibility of the results obtained by a particular method. In good laboratory practice non-standard methods, methods developed internally in laboratories or published in scientific papers, standardized methods with expanded working area and modified standardized methods are validated. In contrast, verification is carried out for standard methods. In verification the laboratory, by carrying out experiments, confirms the appropriate application of the method and the good functioning of laboratory instruments and analysts.

In order to validate and/or verify a method, it is not necessary to determine all performance characteristics, but only those that are important. Thus, for qualitative analysis (identification of sample), it is important to determine accuracy, detection limits, selectivity and robustness. However, in the case of quantification, almost all performance characteristics are determined. At the beginning of validation process it is necessary to define the acceptance criteria for the performance characteristics of the selected method. In practice, however, the most difficult problem for laboratories is setting acceptance criteria based on the performance characteristics of the method, rather than their selection. Criteria should be determined before conducting validation experiments, taking into account the ability to interpret the results obtained by the method. That is to make correct decisions based on these results. The correct setting of criteria and their interpretation requires significant experience of the analyst [14,15]. The quantification of iron ions can be performed using classical analytical methods (gravimetry and volumetry) and various instrumental techniques (UV/Vis spectrophotometry, ICP-OES, ICP-MS, AAS, GF-

AAS, electrochemical, etc.). The choice of method is primarily determined by the concentration range of iron ions in the tested sample and the amount of sample available for analysis, as well as the available equipment. In this work, UV/Vis spectrophotometry with 3-(2-pyridyl)-5,6-bis-(phenylsulfonic acid) 1,2,4-triazine disodium salt (Spectroquant®) as a specific spectrophotometric reagent was chosen as a suitable method. Spectroquant® at pH 3.8 forms a complex with iron, which absorbs at 562 nm.

The proposed method for the determination of iron in cotton materials was developed in-house as a proprietary method. The main purpose of the Spectroquant® reagent is the determination of iron in water samples. Considering a very high selectivity and sensitivity of selected reagent in the determination of iron, the selected method was modified for the determination of iron in cotton materials. For the reasons mentioned above, it is necessary to carry out a validation procedure by determining the performance characteristics [16] in order to test the applicability of the validated method to the analysis of iron in cotton materials after the sample preparation by wet digestion.

2. Experimental

2.1. Validation of spectrophotometric method

Spectrophotometric determination of iron was carried out by addition of 1 cm³ iron standard solution or waters sample, which contains iron, and 10 drops of Spectroquant® reagent (Merck Millipore) into 25 cm³ volumetric flasks with the final addition of distilled water. Absorption spectra are recorded for each sample in region 400-600 nm using UV/Vis spectrophotometer Lambda 20, PerkinElmer. Iron conc. in solution is determined using Lambert-Beer law at wavelength of complex absorption maxima ($\lambda=562$ nm).

Before validation the amount of Spectroquant® reagent was optimized for the concentration range of 0 to 6.5 mg Fe dm⁻³, which is expected in real samples. The spectrophotometric method for iron determination was validated by determining the following performance characteristics. The quantification of iron ions on cotton materials after the wet digestion process was also determined.

Linearity was assessed by calculating the correlation coefficient (R^2) using the linear regression analysis of the calibration curve [14,17]. Calibration curve was obtained by measuring the absorbance of standard iron solutions. Each measurement was made in triplicate.

The standard solutions used in the preparation of the calibration curve were prepared by diluting the standard solution (1000 ± 10 mg Fe dm⁻³, Hach) according to the scheme (Fig.1).



Fig.1 Scheme for preparation of a standard iron solutions series

Using the slope and intercept data the limit of detection (L_D) and limit of quantification (L_Q) were calculated as the product of the standard deviation (σ) multiplied by a factor of 10 and 3.3 and the reciprocal value of the calibration curve slope (S) according to eq. (1) and (2) [14,15].

$$L_Q = 10 \cdot \frac{\sigma}{S} \quad (1)$$

$$L_D = 3,3 \cdot \frac{\sigma}{S} \quad (2)$$

The precision was expressed through the repeatability and intermediate precision. Repeatability was determined by measuring the absorbance at the wavelength of maximum absorption for ten identically prepared standard solutions. Three different concentrations of iron standards within the working range (lowest, middle and highest concentration) were selected for precision testing. Intermediate precision was assessed by measuring the absorbance at three concentrations within the working range by the same analyst, employing both automatic pipettes and conventional glassware. The reproducibility of the method was monitored by identical testing after a period of 3 weeks. UV/Vis spectra were recorded for 10 identical solutions. Statistical parameters were calculated for each set of measurements: mean value, standard deviation (SD) and relative standard deviation (RSD).

The trueness was tested by measuring the absorbance of three standard solutions of different concentrations within the working range and comparing the values with the theoretical absorbance value obtained from the calibration curve.

The robustness was tested by adding a standard copper solution (5, 10 and 20 mg Cu dm⁻³) to the mean concentration within the working range (1.5 mg Fe dm⁻³). The tests were carried out on a set of 10 solutions. The robustness of the method was expressed through statistical indicators: standard deviation (SD) and relative standard deviation (RSD). In the same way, the robustness was tested in the presence of 3 different surfactants: anionic surfactant (sodium dodecyl sulfate, SDS), nonionic surfactant (Triton-X 100) and a combination of alkyl sulfonates and ethoxylates (Kemonecer WET), each in volume fractions of 0.5, 1 and 2%.

2.2. Analysis of cotton materials

Standard cotton fabric samples labeled WFK 11A (according to ISO 2267:2016 bleached, untreated 100% cotton fabric, width 80 cm, with a marking thread in the warp direction) were prepared so that five sub-samples of textile material could be randomly selected and cut. The mass of each sub-sample was between 150 mg and 200 mg (labels 1S-5S).

Sample analysis was carried out by standard addition method. Each subsample was put in reagent bottle containing standard iron solution ($V = 50 \text{ cm}^3$, $\gamma = 10 \text{ mg dm}^{-3}$). The reagent bottles were placed in a Heidolph Unimax 1010 incubator shaker and allowed to mix at 175 rpm at 25 °C for 24 h. Finally, samples 1S-5S were removed into a Petri dish and left in a desiccator overnight to dry.

2.2.1. Sample preparation of the analysis

Cotton fabric samples were prepared for the analysis by wet digestion method. A few drops of concentrated sulfuric acid, H₂SO₄ (96%, Merck Millipore) and hydrogen peroxide solution, H₂O₂ (w = 30%, GRAM-MOL d.o.o.) are added to a material sample in a beaker on a hot plate at a temperature of 350 °C.

In the presence of concentrated sulfuric acid, which has both strong acid, oxidizing and dehydrating properties, cellulose decomposes into carbon (IV) oxide (CO₂) and water (H₂O), which evaporates. The remaining H₂SO₄ decomposes into sulfur (VI) oxide, SO₃ and H₂O at 338 °C. The remaining mineral residue in the form of iron (II) sulfate, FeSO₄, dissolves in distilled water. Depending on the iron content, the solution acquires a more or less intense yellow-brown color, which is an indicator of the presence of Fe³⁺ ions [16], formed by the oxidation

of Fe²⁺ ions. After wet digestion pH value of solution is measured. If pH value was below 3.5, it was adjusted to 3.5 by the gradual addition of NaOH solution (Sigma-Aldrich).

Samples for spectrophotometric determination of iron are prepared as described above. Based on the calibration curve, the concentration of iron ions in the sample is determined. Results are expressed as the mass of iron per mass of material. In addition, the amount of adsorbed iron is indirectly determined by quantifying the residual amount of iron in the solution after adsorption onto the cotton material.

3. Results and discussion

In this work iron on cotton materials was determined after wet digestion sample preparation. Iron content was monitored spectrophotometrically with commercial reagent Spectroquant®. Fe²⁺ ions react with 3-(2-pyridyl)-5,6-bis-(phenylsulfonic acid) 1,2,4-triazine disodium salt (Spectroquant®, Fig.2) as a specific reagent, giving red-violet-colored complex that absorbs at 562 nm. Reagent also contains thioglycolic acid (pH 3.8), as a reducing agent for Fe³⁺. Therefore, this method determines the total iron content.

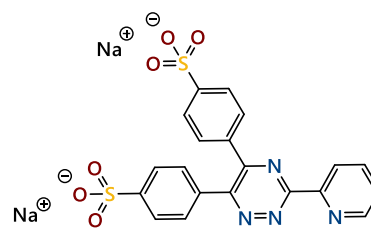


Fig.2 Structure formula of Spectroquant® reagent 3-(2-pyridyl)-5,6-bis-(phenylsulfonic acid) 1,2,4-triazine disodium salt)

According to manufacturer's instructions, the measurement system is prepared by adding 3 drops of Spectroquant® reagent (0.15 cm³) to sample. However, the addition of 0.15 cm³ of Spectroquant® resulted in weakly colored solution, which was confirmed by recording absorption spectra (for $\gamma < 0.2 \text{ mg Fe dm}^{-3}$ $A < 0.1$). Therefore, in further studies, it became necessary to modify the method.

For this reason, the method was optimized with respect to the amount of reagent added. The effect of adding different volumes of reagent (0.15; 0.25 and 0.50 cm³) was tested for the concentration range of 0-6.5 mg dm⁻³ Fe. It was shown that adding 0.15 and 0.25 cm³ of reagent leads to stagnation in absorbance values already at iron concentration of 1 mg dm⁻³. However, adding 0.5 cm³ of Spectroquant® leads to stagnation at iron concentration of 2.5 mg dm⁻³, which gives a wider linear range.

Adding 0.75 and 1.0 cm³ of reagent no longer leads to a significant increase in absorbance values. In addition, at the same time large amounts of reagent are consumed (Fig.3). This effect is a consequence of the fact that, regardless of the increase in iron concentration in the solution, there is not enough reagent to form a complex with the iron ions present. Therefore, in further research as an optimal amount 0.5 cm³ of reagent was chosen.

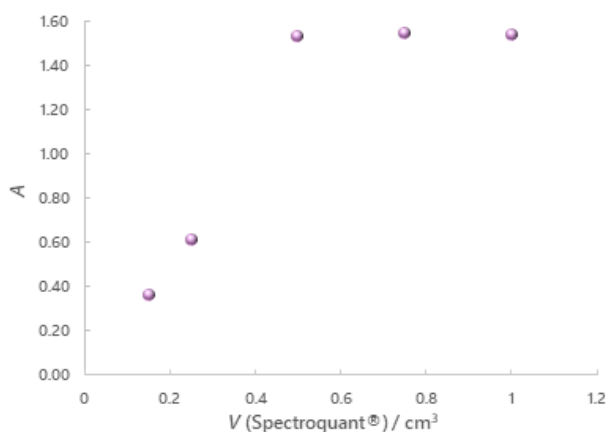


Fig.3 Dependence of the formed complex adsorbance on the amount of Spectroquant® reagent at iron concentration of 10 mg dm⁻³

The spectrophotometric method for iron determination was validated by determining the following performance characteristics: linearity, working range, sensitivity, selectivity, precision (repeatability and interprecision), trueness, limit of quantification and detection, robustness, and estimating the costs of the method. Table 1 presents the chosen performance characteristics along with acceptance criteria, which need to be met in order to validate method.

Tab.1 Selected criteria for validation of a spectrophotometric method for the determination of iron in aqueous solutions using Spectroquant® reagent

Performance characteristics	Criteria
Linearity	R ² ≥ 0,998
Limit of detection	Information
Limit of quantification	Information
Working range	Information
Repeatability	< 10%
Intermediate precision	< 10%
Reproducibility	< 10%
Trueness	90-100%
Robustness	90-110%

Linearity is defined as a possibility of the method to provide responses proportional to the concentration of the analyte present in the sample within a defined concentration range [14, 15]. For this purpose, the

absorbance ratio for standard iron solutions in the concentration range of 0.01–5.0 mg Fe dm⁻³ was monitored with the addition of 0.5 cm³ of Spectroquant® reagent. A calibration curve was constructed by linear regression of the measured experimental points. It illustrates the dependence of the absorbance of the formed Fe-Spectroquant® complex on iron concentration (Fig.4).

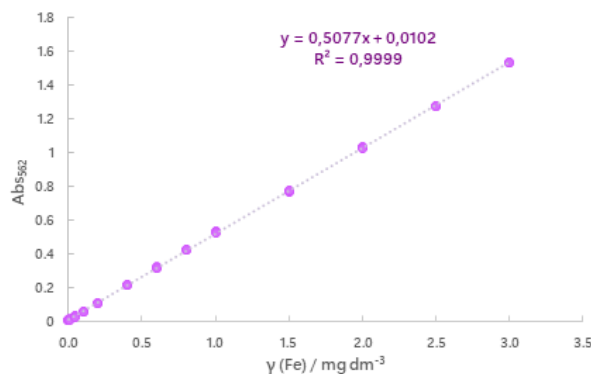


Fig.4 Calibration curve for spectrophotometric determination of iron in the concentration range 0.16-3.0 mg Fe dm⁻³

The correlation coefficient for the concentration range is 0.9999. The calculated value satisfies the criteria R² > 0.99. Therefore, the linear dependence of absorbance A on the iron mass concentration γ(Fe) is confirmed. The calibration curve's equation has an intercept on the ordinate with a value of 0.0102. It can be considered that a systematic error has been introduced into the measurement system, which can be caused by an error in the instrument, method or analyst. However, the value of the intercept is very small. So, it can be considered negligible and is certainly smaller than the limits prescribed by the criteria (Tab.1).

The linearity examination showed that the dependence of the complex absorbance on the iron concentration is linear up to 3 mg Fe dm⁻³. At higher iron concentrations there is insufficient reagent in the solution to form an equivalent amount of the complex. Moreover, at high concentrations (A > 2), changes in molar absorptivity (ε) occur, mainly as a result of the interaction of absorbing species. Therefore, further expansion of the working range is not recommended. The upper limit of the working range is set at a concentration of 3 mg Fe dm⁻³. Regulation on limit values for wastewater emissions (NN 26/2020) stipulates that surface waters can support iron concentrations of up to 2 mg dm⁻³. Therefore, the maximum allowable concentration (MAC) value will be within the working range of the method. However, water discharged into public drainage systems can support concentrations of up to 10 mg dm⁻³, which are outside the working range of this method. Therefore,

if a sample of such waters is analyzed, it will be necessary to make a prior dilution in order for the tested concentration to be within the working range. Due to the lack of information on possible MAC values of iron on cotton materials, the maximum expected values of iron on the material in this study were defined through the MAC values of process waters used in textile finishing processes.

Parameters lower limit of detection (L_D) and limit of quantification (L_Q) were calculated according to eq. (1) i (2). Determined L_D and L_Q are 0.05 and 0.16 mg Fe dm⁻³. Limit of quantification represents the minimum quantity of an analyte in a sample with can be quantified with corresponding precision and accuracy. So 0.16 mg Fe dm⁻³ was set up as lower limit of working range.

Accuracy represent the degree of agreement between experimental results and an accepted reference value. Experimental results are considered accurate if they do not differ significantly from the true value. In other words, an accurate method is one that allows obtaining precise results without the influence of systematic error [14,15]. Standards for laboratory quality systems (e.g. ISO standards) require that results be metrologically traceable, which is ensured by the use of reference materials (RM), or certified reference materials (CRM). Reference materials are samples of certain properties that accurately represent a real sample with respect to the chemical composition of the analyte and its matrix. In this paper, accuracy is determined by determining precision and trueness, because CRM and RM for the determination of iron in cotton textile materials do not exist.

Precision of the method is defined as the agreement of a series of measurements performed from the same homogeneous sample under defined conditions [14, 15]. In this work the precision was expressed through repeatability and intermediate precision, and reproducibility, with numerical precision indicators provided. Three concentrations were tested covering the entire working range: 0.1; 1.0 and 2.0 mg Fe dm⁻³ (Tab.2). From the obtained experimental data and the calibration curve the actual iron concentration in the prepared iron standard solutions was determined. It was found that the actual iron concentration in the standard solution was slightly lower than the expected (prepared) one. This is primarily attributed to systematic error (pipetting error), but also to imperfections of the measuring devices (automatic pipettes and UV/Vis spectrophotometer). A more detailed insight into the sources of systematic error can be obtained by assessing the measurement uncertainty of each component of the method, which is not the subject of this paper. The obtained precision indicator values indicate a very small dispersion of the experimental

data. The acceptance criterion for determining precision is defined depending on the expected conc. range of the analyte [14,15]. It follows that for an analyte content of 1 mg dm⁻³ the allowed RSD is 10%, while for a concentration of 0.1 mg dm⁻³ an error of as much as 20% is allowed. From the results shown in Table 2 for the repeatability test it is clear that the selected method meets the acceptance criterion. Intermediate precision tests have shown that the RSD values when using glass measuring vessels are somewhat higher compared to the use of automatic micropipettes, especially at lower concentrations. Therefore, the use of automatic micropipettes will be preferred for routine analyses due to precision and the speed of analysis. Also, the reproducibility tests met the set acceptance criteria. The differences in the obtained values can be attributed to systematic errors due to changes in atmospheric pressure and temperature and the stability of chemicals.

Tab.2 The precision of method for spectrophotometric determination of iron in water samples

γ (Fe) / mg dm ⁻³	REPETABILITY		
	γ (Fe)= 0.1 mg dm ⁻³	γ (Fe)= 1.0 mg dm ⁻³	γ (Fe)= 2.0 mg dm ⁻³
	0.097±0.0079 (RSD=8.14%)	0.968±0.0076 (RSD=0.78%)	1.9444±0.01384 (RSD=0.71%)
	INTERMEDIATE PRECISION		
	γ (Fe)= 0.12 mg dm ⁻³	γ (Fe)= 1.2 mg dm ⁻³	γ (Fe)= 2 mg dm ⁻³
	0.110±0.0106 (RSD=9.6%)	1.171±0.0126 (RSD=1.1%)	1.991±0.0266 (RSD=1.3%)
	REPRODUCIBILITY		
	γ (Fe)= 0.1 mg dm ⁻³	γ (Fe)= 1.0 mg dm ⁻³	γ (Fe)= 2.0 mg dm ⁻³
	0.094±0.0122 (RSD=13.0%)	1.002±0.0270 (RSD=2.7%)	2.028±0.0181 (RSD=0.9 %)

Trueness was tested for 3 concentrations within the working range (0.25; 1.25 and 2.75 mg Fe dm⁻³). Since there is no certified reference material for the determination of iron on cotton material, a theoretical value was calculated based on the obtained sensitivity of the calibration curve. According to the defined acceptance criteria, the recovery should be in the range of 90-110% [14]. The results confirm the accuracy of the measurements, according to which the recovery for the tested concentrations took values in the range of 95-100%.

Robustness is defined as a measure of the resistance of an analytical procedure to small, unintended changes in operating conditions. It provides information on the reliability of the method during its use. Robustness testing reveals the optimal operating conditions of the method and the parameters that need to be monitored [14,15]. According to the manu-

facturer's instructions, a number of substances interfere with the determination of iron using the aforementioned reagent. These include Al^{3+} , Ca^{2+} , Cd^{2+} , CN^- , Co^{2+} , Cr^{3+} , $Cr_2O_7^{2-}$, Cu^{2+} , Hg^{2+} , Mg^{2+} , Mn^{2+} , MoO_4^{2-} , NH_4^+ , Ni^{2+} , NO_2^- , Pb^{2+} , PO_4^{3-} , SiO_3^{2-} , Zn^{2+} , EDTA, surfactants, Na-Ac, NaCl, $NaNO_3$ and Na_2SO_4 . Water from public drainage systems, usually used in textile washing processes, contains calcium and magnesium. However, the concentrations are much lower than those that would cause interference in iron determination ($100 \text{ mg Ca dm}^{-3}$ and 20 mg Mg dm^{-3}). During the determination of iron content on cotton textiles, the interference may be caused by:

- (1) Cu^{2+} ions, which usually come in combination with iron ions [10]
- (2) Surfactants, as the main components of detergents used in the washing and care of textiles [18].

According to the specifications of the reagent manufacturer, a copper concentration of 10 mg dm^{-3} causes interference by increasing the absorbance values. That is, give an apparent increase in iron concentrations. In this work, the interference due to the presence of copper ions at concentrations of 5; 10 and 20 mg Cu dm^{-3} was examined. It can be confirmed that the method is robust, i.e. resistant to changes in the presence of a Cu^{2+} solution with the RSD not exceeding the limit of 15% and the recovery not exceeding the upper limit of 110% (Table 3).

Tab.3 The influence of Cu^{2+} ions ($\gamma = 5; 10$ and 20 mg dm^{-3}) on the spectrophotometric determination of iron in water samples

	$\gamma (Cu) = 5 \text{ mg dm}^{-3}$	$\gamma (Cu) = 10 \text{ mg dm}^{-3}$	$\gamma (Cu) = 20 \text{ mg dm}^{-3}$
$\gamma (Fe) / \text{mg dm}^{-3}$	1.534 ± 0.0092 (RSD= 0.60%)	1.584 ± 0.0067 (RSD = 0.43%)	1.612 ± 0.0138 (RSD = 0.86%)
I [%]	100.89	104.19	106.02

The manufacturer states that interference occurs in the presence of anionic, cationic and nonionic surfactants in concentrations greater than 1%. At the same time, the presence of surfactants in the textile wastewater, especially from laundries, can have a negative effect by increasing the solubility of iron ions in water in the form of complexes. Formed complexes are then additionally deposited on the material and cause further damage [18]. The influence of the presence of three commonly used surfactants on the determination of iron using the Spectroquant® reagent (Table 4) was tested:

- (1) Anionic surfactant - sodium dodecyl sulfate (SDS)
 - (2) Nonionic surfactant - Triton X-100 (TX),
 - (3) Kemonezer WET (KnW), a combination of alkanesulfonates and ethoxylates,
- and the results of validation are shown in Table 5.

Tab.4 The influence of sodium dodecyl sulfate (SDS), Triton X-100 (TX) and Kemonezer WET (KnW) surfactants ($w = 0.5, 1$ and 2%) on the spectrophotometric determination of iron in water samples

$\gamma (Fe) / \text{mg dm}^{-3}$			
	$w (SDS) = 0.5 \%$	$w (SDS) = 1 \%$	$w (SDS) = 2 \%$
	1.584 ± 0.0427 (RSD= 2.69%)	1.551 ± 0.0214 (RSD= 1.38 %)	1.525 ± 0.0073 (RSD= 0.48%)
I [%]	104.20	102.03	100.29
	$w (TX) = 0.5 \%$	$w (TX) = 1 \%$	$w (TX) = 2 \%$
	1.573 ± 0.0169 (RSD= 1.07 %)	1.558 ± 0.0128 (RSD= 0.82%)	1.562 ± 0.0088 (RSD= 0.56 %)
I [%]	100.34	99.40	99.62
	$w (KnW) = 0.5 \%$	$w (KnW) = 1 \%$	$w (KnW) = 2 \%$
	1.522 ± 0.0189 (RSD= 1.24%)	1.557 ± 0.0213 (RSD= 1.37%)	1.551 ± 0.0142 (RSD= 0.91%)
I [%]	98.15	100.37	100.00

Tab.5 The results of validation of spectrophotometric method for determination of total iron content in water samples

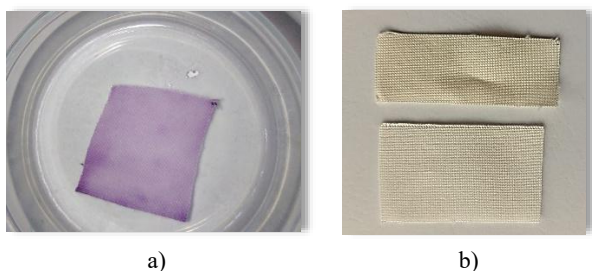
Performance characteristics	Criteria	Results
Linearity	$R^2 \geq 0.998$	$A = 0.5077 \gamma (Fe) + 0.0102$; $R^2 = 0.9999$
Limit of detection	information	$0.05 \text{ mg Fe dm}^{-3}$
Limit of quantification	information	$0.16 \text{ mg Fe dm}^{-3}$
Working range	information	$0.16 - 3.00 \text{ mg Fe dm}^{-3}$
Repetability	$< 10\%$	$0.1 \text{ mg Fe dm}^{-3}$ (8.14%); $1.0 \text{ mg Fe dm}^{-3}$ (0.78%); $2.0 \text{ mg Fe dm}^{-3}$ (0.71%)
Intermediate precision	$< 10\%$	$0.12 \text{ mg Fe dm}^{-3}$ (9.6%); $1.2 \text{ mg Fe dm}^{-3}$ (1.1%); $2.0 \text{ mg Fe dm}^{-3}$ (1.3%)
Reproducibility	$< 10\%$	$0.1 \text{ mg Fe dm}^{-3}$ (13.0%); $1.0 \text{ mg Fe dm}^{-3}$ (2.7%); $2.0 \text{ mg Fe dm}^{-3}$ (0.9%)
Accuracy	90-100%	$0.25 \text{ mg Fe dm}^{-3}$ (95.6 %); $1.25 \text{ mg Fe dm}^{-3}$ (99.0%); $2.75 \text{ mg Fe dm}^{-3}$ (99.4%)
Robustness	90-110%	$5 \text{ mg Cu}^{2+} \text{ dm}^{-3}$ (100.89%); $10 \text{ mg Cu}^{2+} \text{ dm}^{-3}$ (104.19%); $205 \text{ mg Cu}^{2+} \text{ dm}^{-3}$ (106.2%); 0.5% SDS (104.2%); 1% SDS (100.3%); 2% SDS (98.2%); 0.5% TX (102.0%); 1% TX (99.4%); 2% TX (100.4%); 0.5% KnW (100.3%); 1% KnW (99.6%); 2% KnW (100.0%)

Results confirm that the method is robust, i.e. resistant to changes in the presence of non-ionic and anionic surfactants and their combinations at volume fractions of 0.5; 1 and 2% (Table 4). This indicates that the recovery is within the allowed range, i.e. the defined criteria of 90-110%. The RSD values are slightly higher compared to those obtained in the tests with the standard Cu solution. These deviations result from surfactant-induced foaming, which hinders the accurate preparation of standard solutions and consequently introduces error into the analytical system.

In order to highlight the fact that the validation of an analytical method is an extremely long and expensive process, the price of validation for variable costs has been approximately calculated. According to the cost estimate for the entire validation procedure, more than €900 would be spent. At the same time, a large amount of expensive reagent was used in this validation procedure to assess whether the selected method produces results with satisfactory statistical parameters. Of course, this estimate does not include fixed costs such as depreciation of the instrument, service and other materials, which would ultimately make the validation procedure itself and the total price of an individual analysis significantly more expensive. For this reason, it is important to find a compromise between the costs and the purpose of validation, taking into account the time spent on method validation.

3.1. Material analysis

Prior to quantitative determination of iron content on standard cotton fabric, a qualitative test was performed to determine whether the sample itself contained adsorbed iron. The test was performed by adding a few drops of Spectroquant® reagent to the material itself. Results (Fig.5a) showed that material ever before addition of standard iron solution contained a certain amount of adsorbed iron. It is for this reason that it is extremely important to conduct a blank analysis for determination of iron content on cotton materials.



SI.5 a) Qualitative test for determining the presence of iron on standard cotton fabric; b) cotton fabric sample after iron adsorption

Results of the blank analysis (Table 6) confirmed that the sample of untreated cotton fabric contained certain amount adsorbed iron. Furthermore, the determined amount of iron in randomly selected blank subsamples varies considerably, ranging from 1.3 - 5.8 mg Fe per gram of material with an RSD greater than 60%. Such deviations clearly indicate a highly heterogeneous distribution of iron onto standard cotton fabric. Heterogeneous distribution of iron originates from the natural iron present in the fiber, but is also a consequence of inappropriate storage of the material as well as the use of chemicals, which contain iron, in finishing processes. After iron adsorption onto the material, yellowing of the material was observed. This is a qualitative indicator that iron has bound to the material itself and created latent damage [19]. Results of samples analysis after adsorption process (Table 6) again confirmed that iron can not be uniformly bound to cotton material. Determined amounts of iron take on values of 3.9-14.3 mg Fe per gram of material.

Tab.6 Results of spectrophotometric determination of iron after wet digestion of untreated sample and sample with adsorbed iron, as well as iron remaining in solution after the adsorption process

Oznaka uzorka	mg (Fe) / g (uzorka)
Fe cott. tex SP-1	4.2732
Fe cott. tex SP-2	5.7747
Fe cott. tex SP-3	1.2919
	3.8±2.28 (RSD = 60.4%)
Fe cott. tex 1S	3.8728
Fe cott. tex 2S	12.2257
Fe cott. tex 3S	9.1881
Fe cott. tex 4S	4.6567
Fe cott. tex 5S	14.2953
	8.8±4.57 (RSD = 51.6%)
Fe cott. tex AQ-1	16.1221
Fe cott. tex AQ-2	10.0201
Fe cott. tex AQ-3	14.7211
Fe cott. tex AQ-4	11.3000
Fe cott. tex AQ-5	12.4303
	12.9±2.49 (RSD = 19.3%)

The amount of adsorbed amount of iron was also determined indirectly by quantifying the residual iron in the solution after the adsorption process. It differs from the amount of iron determined by the wet combustion process, and mostly assume higher values. It is assumed that during adsorption process the iron is continuously adsorbed onto material and desorbed into solution. Depending on surface characteristics of materials iron ions can be more bind or released. Considering the fact that iron is manly bind onto cellulose though ion exchange mechanism, the dynamic equilibrium that is established in this process should also be taken into account. In addition, the wet digestion is performed in open system. So due to the

exothermic reaction, there is certainly a loss of substance. Consequently, a smaller amount of iron is quantified after wet combustion compared to direct determination via the residual amount of iron in solution. Therefore, in future research it is necessary to compare the efficiency and recovery of alternative methods of sample preparation in closed systems when there is no loss of substance (e.g. microwave digestion).

Although there is a large deviation of experimental data, the smallest of them are observed in the solution samples. This is exactly the working area covered by the validation of the method. Such large deviations in the results again point to the fact that textile materials have very heterogeneous structure. For this very reason, a homogeneous distribution of iron along the entire surface of the cotton textile material cannot occur. In future research, the so-called "mapping" of standard materials should be carried out. Though out "mapping" the range of expected concentrations of iron adsorbed on the standard material, along with the calculated statistical parameters, would be determined. According to the literature [12], precisely in the areas with locally increased amounts of iron the degradation during washing processing in alkaline baths can occur.

4. Conclusions

In this paper, an improved methodology for the determination of iron ions on cotton materials is proposed. The iron ions present on textiles after wet digestion were monitored spectrophotometrically using 3-(2-pyridyl)-5,6-bis-(phenylsulfonic acid) 1,2,4-triazine disodium salt (Spectroquant®) as a specific reagent for iron. Since this is a modified method for the determination of iron in aqueous samples, the method for the quantification of iron ion concentration on cotton materials was validated.

Based on the conducted experiments, the following conclusions can be made:

- The validation determined that the selected reagent is suitable for the quantification of iron ions in water in a wide range of concentrations (0.16-3.00 mg Fe dm⁻³) with a low limit of detection (0.05 mg Fe dm⁻³) and quantification (0.16 mg Fe dm⁻³), a high level of precision and accuracy (RSD < 10%). At the same time, the presence of Cu²⁺ and various surfactants, which are very often found in textile wastewater, will not cause significant interferences in the determination of iron.
- A qualitative test on a cotton fabric sample using the Spectroquant® reagent indicated that the

observed standard cotton materials contain pre-adsorbed iron. Thus an improved protocol was established according to which the analysis of the standard material is carried out after the adsorption process from the standard iron solution with mandatory simultaneous analysis of a blank sample.

- The discrepancy in the results of the indirect and direct determination of iron in cotton textile material confirmed that there is a dynamic equilibrium in the process of ion exchange of iron bound to the material and that remaining in the solution. Taking into account the heterogeneity of the material, it is not possible to unambiguously determine the amount of iron on the cotton material. Instead, the range of expected concentrations with the indicated statistical parameters can be defined.
- Wet digestion for the sample preparation is performed in an open system. This results in a certain loss of material, which ultimately affects the measured iron content compared to the expected value. Therefore, in future research, the results obtained in this way should be compared with the sample preparation method in a closed system (e.g. microwave digestion), in which there would be no loss of the sample.
- Large discrepancies in the results again point to the fact that textiles are materials with a very heterogeneous structure. This is why iron cannot be uniformly adsorbed, or bound by ion exchange, throughout the entire material.

Future research should also include the so-called "mapping" of standard materials, which would determine the range of expected concentrations of iron adsorbed on the standard material, along with the calculated statistical parameters.

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