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Short communication

Selective determination of Fe(III) in Fe(II) samples by UV-spectrophotometry with the aid of quercetin and morin

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Selective UV-spectrophotometric methods for determination of iron(III) in iron(II) samples have been developed. The methods are based on the interaction of Fe(III) with quercetin and morin, compounds of the flavonoid group. Redox reactions occurring between Fe(III) ions and the reagents used make the basis for the detection. Iron(II) does not react with quercetin and morin under the conditions applied [aqueous-methanolic (3:2) soluions, 0.3 mol L^{-1} HCl, 1.2×10^{-4} mol L^{-1} quercetin (morin)] and does not interfere with the determination of Fe(III). Iron (III) can be determined up to 15 μg mL^{-1} using both the examined systems. The detection limits are 0.06 and 0.38 μg mL^{-1} when using quercetin or morin, respectively. The method with quercetin was applied to the determination of Fe(III) (*ca.* 0.2%) in a Fe(II) pharmaceutical product.

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Iron is one of the most important essential elements. Its deficiency or overload may cause health problems. Speciation of iron, occurrence of the element in two oxidation states (II and III) and equilibrium between these forms are important for biological systems using iron for metabolic processes. Different biological activities of Fe(II) and Fe(III) are well known (1). Iron(II) is favoured for absorption by biological cells. In case of clinical symptoms of iron deficiency some medicaments containing Fe(II) can be administrated to eliminate complications. Low stability of Fe(II) caused by its easy oxidation to Fe(III) by air oxygen can result in a decrease of the real Fe(II) concentration in pharmaceuticals. Examination of the occurrence of Fe(III) in Fe(II) supplements is important.

In this work, simple spectrophotometric methods have been developed for determination of Fe(III) in Fe(II) samples. The methods use selective reaction of Fe(III) with two flavonoid compounds, quercetin and morin. Flavonoids exhibit chelating properties to metal ions. Chelation is accomplished by carbonyl and hydroxyl groups present in flavonoid molecules (Fig. 1). The complexes formed make the basis for determination of metals by various instrumental techniques (2, 3).

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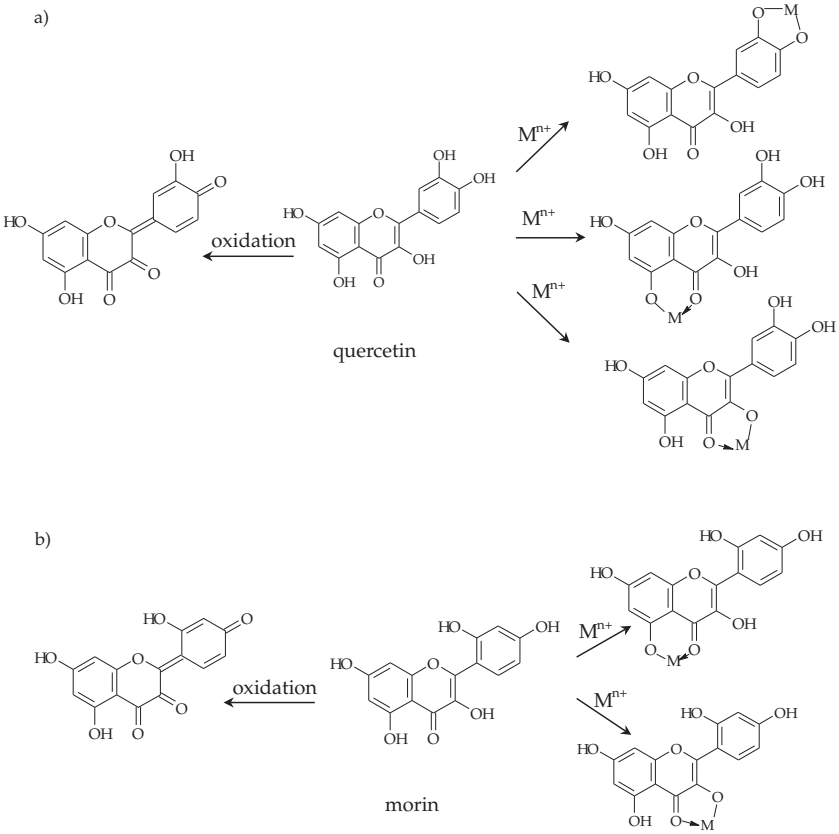

Fig. 1. Structures of: a) quercetin and b) morin, and the products of their reactions with metal ions.

Besides metal chelation capabilities, flavonoids exhibit reducing properties (*via* electron or H-atom donation) (4, 5). Quinones are suggested as flavonoid oxidation products. Occurrence of redox reactions has been observed in the systems containing metal ions and flavonoid compounds (6–12). In our earlier studies (9, 11, 12), we proved that the oxidized forms of quercetin and morin generated in the presence of ruthenium(IV), gold(III) and osmium(VIII) could be successfully used for quantification of metals in the examined solutions. In this work, the redox reaction of Fe(III) with quercetin has been used for its determination in Fe(II) samples.

EXPERIMENTAL

Reagents

Iron(III) standard solution (1 mg mL^{-1}) was prepared by dissolving iron(III) chloride ($FeCl_3$, Merck-Schuchardt, Germany) in 0.1 mol L^{-1} HCl. Iron(II) standard solution (1 mg mL^{-1}) was prepared by dissolving iron(II) sulphate ($FeSO_4 \times 7H_2O$, Fluka Chemika, Switzerland) in 0.1 mol L^{-1} HCl. Quercetin standard solution, 1×10^{-3} mol L^{-1}, was prepared by dissolving 3,5,7,3′,4′-pentahydroxyflavone $\times 2H_2O$ (Sigma-Aldrich, Germany) in methanol. Morin standard solution, 1×10^{-3} mol L^{-1}, was prepared by dissolving 2′,3,4′,5,7-pentahydroxyflavone (Sigma-Aldrich) in methanol.

Pharmaceutical formulation

Fe(II) supplement (Ferro-Gradumet, Abbot Laboratories, UK) was examined for Fe(III) content. According to the producer, the product contained 325 mg of Fe(II) sulphate [105 mg of Fe(II)] per tablet.

Apparatus

Absorbance was measured and absorption spectra were recorded using a JASCO V-560 (Japan) UV-VIS spectrophotometer. Quartz cells (l = 1 cm) were used.

Procedure

To the test solution containing not more than 150 μg Fe(III) and 45 mg Fe(II), the following were subsequently added: 1.2 mL of 1×10^{-3} mol L^{-1} quercetin (or 1.4 mL of 1×10^{-3} mol L^{-1} morin), 3 mL of methanol and hydrochloric acid in such amount so as to obtain a final 10-mL volume of 0.3 mol L^{-1} HCl. The sample was diluted to 10-mL with 0.01 mol L^{-1} HCl and heated at 70 °C for 20 min. The solution was cooled and its volume was adjusted to 10-mL with 0.3 mol L^{-1} HCl in case of evaporation. Fe(III) concentration was determined using absorbance at 291 nm (quercetin method) and 293 nm (morin method) and the appropriate regression equation.

RESULTS AND DISCUSSION

Reaction of Fe(III) with quercetin

The experiments have shown that both redox and complexation reactions can simultaneously occur in solutions containing Fe(III) in hydrochloric acid medium and quercetin. Aqueous-methanolic (3:2) solutions were used because of limited solubility of quercetin in pure water. Formation of two species exhibiting absorption bands in visible and UV regions (Fig. 2a – curves 3 and 3′, respectively) has been observed. The absorption band in the visible region is characteristic of the complex of the metal ion with the reagent. Maximum absorbance of the complex of Fe(III) with quercetin occurs at λ_{max} =

422.5 nm. The second absorption band in the 260–340 nm region is characteristic of the oxidized form of the reagent. Similar absorption bands, identified as characteristic of quinone derived from the flavonoid compounds used, were observed earlier in the other systems containing quercetin and morin and metal ions of oxidizing properties, Ru(IV), Au(III) and Os(VIII) (9, 11, 12).

Equilibrium of both complexation and redox reactions, as well as the amount of particular species formed depend on the conditions applied: hydrochloric acid concentration, reaction time and temperature. Lower acid concentrations are favourable for formation of the Fe(III)-quercetin complex. Increase in HCl concentration in the solution containing the complex results in shifting the equilibrium into the redox process and quantitative formation of the final oxidized form of quercetin (quinone) with λ_{max} at 291 nm (Fig. 2a – curve 4). Optimum HCl concentrations for the formation of the complex and product of the redox reaction amount to 0.03 mol L^{-1} and 0.3 mol L^{-1}, respectively.

In our experiments we observed low stability of the Fe(III) complex with quercetin. Rapid decrease (*ca.* 98% within 1 h) of its absorbance was recorded (Fig. 3 – curve 1). Simultaneous increase in the amount of species with λ_{max} at 291 nm was observed (Fig. 3 – curve 2). This confirms the occurrence of the redox reaction between Fe(III) and quercetin with time. The process can be significantly accelerated by heating the solution. Quantitative formation of the redox reaction product and complete decomposition of the complex was observed after 15 min of heating at 70 °C. Longer heating time under the conditions used has no effect on the equilibrium of the reaction examined.

The final redox reaction product (λ_{max} at 291 nm) is stable. No changes in its absorption spectrum was observed within 5 days. The experiments have shown that this product can be used for quantification of Fe(III). Its amount depends on the Fe(III) concentration in the examined solution. The solutions obey Beer's law in the range of 0.1–15 μg mL^{-1} Fe(III). The molar absorptivity (ε) equals 7.5×10^3 L mol^{-1} cm^{-1}. The regression equation obtained for the solutions containing Fe(III) in 0.3 mol L^{-1} HCl and 1.2×10^{-4} mol L^{-1} quercetin (established as optimum) after 15 min of heating at 70 °C is $y = 0.1271\gamma$

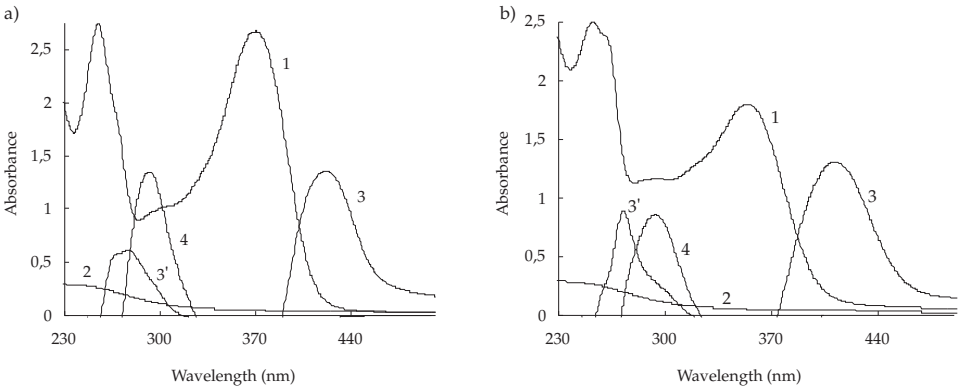

Fig. 2. Spectra of: a) quercetin [1.2×10^{-4} mol L^{-1} (curve 1)], and b) morin [1.4×10^{-4} mol L^{-1} (curve 1)] and the products of their reaction with Fe(III) [10 μg mL^{-1} in 0.3 mol L^{-1} HCl (curve 2)]: directly after mixing the reagents (curves 3 and 3′) and after heating for 20 min at 70 °C (curve 4).

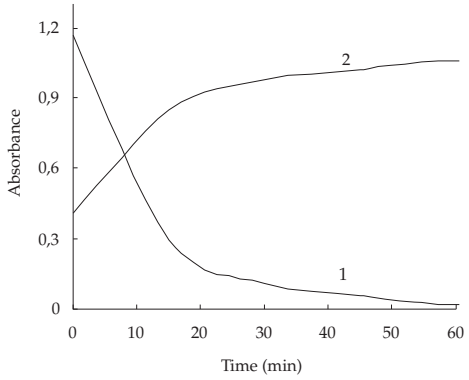

Fig. 3. Changes in the absorbance of the Fe(III)-quercetin complex at λ_{max} 422.5 nm (curve 1) and the redox reaction product at λ_{max} 291.0 nm (curve 2) in time.

+ 0.0572, where y is absorbance at 291 nm and γ is the concentration (μg mL^{-1}) of Fe(III) ions, coefficient of determination R^2 = 0.9946. Detection limit estimated from the standard deviation (SD) of the blank and calibration sensitivity (slope of calibration line) [*LOD* = 3:3 SD/sensitivity (13)] amounted to 0.07 μg mL^{-1} Fe. The relative standard deviation (RSD) of the results of the determination of Fe (50–150 μg in 10 mL solutions) was in the range of 0.1–0.7% (Table 1).

Under the optimum conditions for Fe(III) determination, no redox or complexation reaction of Fe(II) with the reagent was observed. We did not observe any interference of Fe(II) with Fe(III) detection up to 300-fold mass excess of Fe(II) to Fe(III) examined in the work. As a consequence, we have drawn the conclusion that the redox reaction occurring in the Fe(III)-quercetin system can serve as the basis for a selective spectrophotometric method for Fe(III) in the presence of Fe(II) ions.

Table I. Determination of Fe(III) with quercetin and morin

Iron(III) (μg)		
Added	Determined[a] (confidence limits, α = 0.05)	RSD (%)
Quercetin method		
50.00	49.74 ± 0.07	0.1
100.00	99.60 ± 0.25	0.2
150.00	148.52 ± 1.04	0.7
Morin method		
50.00	49.85 ± 0.09	0.2
100.00	98.49 ± 0.67	0.6
150.00	148.22 ± 0.61	0.4

[a] Mean ± SD (n = 6).

Reaction of Fe(III) with morin

The experiments have shown that morin (Fig. 2b) reacts very similarly to quercetin with Fe(III) ions. Also, the complexation and redox reactions occur in methanolic-aqueous solutions containing Fe(III) and the reagent. Equilibrium between the reaction products mainly depends on the hydrochloric acid concentration in the examined samples. Shifting to the formation of the Fe(III) complex with morin in less acidic media (0.03 mol L^{-1} HCl as optimal) (Fig. 2b – curve 3) and to the redox reaction product in solutions of higher HCl concentration (Fig. 2b – curve 4) has been observed. The solution of 0.3 mol L^{-1} HCl was found optimal for quantitative reduction of Fe(III) ions by morin. The redox reaction occurs with time. Complete reduction of Fe(III) was observed after 15 min heating of the solution at 70 °C.

The complex of Fe(III) with morin (λ_{max} = 411.5 nm) exhibits low stability, similarly to that with quercetin. About 60% decrease in its absorbance has been observed within 1 hour. Rapid, complete decomposition of the complex occurs under heating of the solution. Contrary to the complex, the oxidized form of morin (λ_{max} = 293 nm), being the product of the redox reaction with Fe(III) ions, is stable. No changes in its spectrum were observed within 5 days. The amount of oxidized morin corresponds to the Fe(III) concentration in the examined solution. Such format can be used for spectrophotometric determination of Fe(III). Molar absorptivity at λ_{max} 293 nm equals 4.8×10^3 L mol^{-1} cm^{-1}. The solutions obey Beer's law in the range of 0.4–15 μg mL^{-1} Fe. The regression equation is $y = 0.0875\gamma + 0.0087$, where y is the absorbance of determination at 293 nm, and γ is the concentration (μg mL^{-1}) of Fe(III) ions with coefficient of determination R^2 = 0.9997. *LOD* amounts to 0.42 μg mL^{-1} Fe. Relative standard deviation of the results of Fe(III) determination was in the range of 0.2–0.7% (Table I).

Similarly as in the case of quercetin, no interaction of Fe(II) with morin was observed under the conditions applied. Large amounts of Fe(II) ions (300-fold mass excess of Fe(II) over Fe(III) examined) did not affect the reaction of Fe(III) with morin.

Determination of Fe(III) in a Fe(II) pharmaceutical formulation

The redox reaction occurring between Fe(III) and quercetin was applied to the determination of Fe(III) in a Fe(II) pharmaceutical product used as an iron supplement for humans. Small amounts of Fe(III) in such products can originate from the oxidation of Fe(II) by air oxygen. Quercetin method was chosen for the determination because of its lower limit of detection.

The tablets (five tablets were taken for analysis) were preliminarily treated with methanol (5 mL for 5 min) to eliminate the protective external layer containing polymeric additives and pigments. After separation of the methanolic extract, the tablets were treated with 5 mL 1 mol L^{-1} HCl. After 20 min, the samples were filtered into a 10-mL volumetric flasks and the obtained solution was diluted to the final volume with 1 mol L^{-1} HCl. The content of Fe(III) was determined in an aligment of 2 mL. The amount of Fe(III) was in the range of 0.210–0.262 mg per tablet (0.234 ± 0.021 mg, n = 6). RSD of the results equaled 9.1%. The amount of Fe(III) determined in the Fe(II) supplement examined was *ca.* 0.2% per tablet. The accuracy of the results was evaluated by the standard addition method. The spike of the standard solution of Fe(III) was introduced into the

examined sample prior to its dissolution in hydrochloric acid. The 96.0 ± 0.5% recovery of the spike was reached.

CONCLUSIONS

Iron(III) reacts with quercetin and morin with simultaneous formation of two products: the respective complexes and oxidized forms of the reagents used. The complexes formed exhibit low stability. Contrary to the complexes, the products of the redox reactions are stable. Such products (λ_{max} at 291–293 nm) can be used for the quantification of iron(III) by UV-spectrophotometry.

No interference from Fe(II) with the detection of Fe(III) was observed for either reagent. This recommends quercetin and morin as selective reagents for the determination of Fe(III) in the presence of large excess of Fe(II). The system with quercetin was applied to the determination of Fe(III) (*ca.* 0.2%) in Fe(II) supplementary drugs. The obtained results confirm the oxidation process of Fe(II) to Fe(III) during storage.

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S A Ž E T A K

Selektivno određivanje Fe(III) u uzorcima Fe(II) UV-spektrofotometrijom pomoću kvercetina i morina

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U radu je opisan razvoj selektivnih UV-spektrofotometrijskih metoda za određivanje željeza(III) u uzorcima željeza(II). Metode se temelje na redoks reakciji Fe(III) sa spojevima iz skupine flavonoida – kvercetinom i morinom u reakcijskim uvjetima u kojima željezo(II) ne reagira (vodeno/metanolna otopina 3:2, 0,3 mol L^{-1} HCl, 1,2 x 10^{-4} mol L^{-1} kvercetin ili morin). Najniža koncentracija željeza(III) koja se može odrediti u oba ispitivana sustava je 15 μg mL^{-1}. Granice detekcije su 0,06 i 0,38 μg mL^{-1} ako se koristi kvercetin odnosno morfin. Metoda s kvercetinom primijenjena je za određivanje Fe(III (približno 0,2%) u farmaceutskom proizvodu Fe(II).

Ključne riječi: željezo, kvercetin, morin, UV-spektrofotometrija

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