

# New sulfonic derivatives of quercetin as complexing reagents: synthesis, spectral, and thermal characterization

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**Abstract** The synthesis conditions of quercetin-8-sulfonic acid and sodium salt of sulfonated quercetin were determined. The molecular formulae of obtained compounds are:  $C_{15}H_{10}O_{10}S \cdot 4H_2O$  and  $C_{15}H_9O_{10}SNa \cdot 4H_2O$ . On the basis of spectral methods UV–Vis, IR, and  $^1H$  NMR it was stated that the sulfonation of quercetin occurs at 8-C position of the A ring. The complexing properties of NaQSA-8 were confirmed by synthesis of a solid complex with Fe(II) ions. The NaQSA-8 formed a complex with Fe(II) ions at pH 5 with the metal excess ( $c_M:c_L = 7:1$ ). The chelation site was analyzed using spectroscopy methods. It was found that chelation of the metal ions occurs at the 4C=O, 3-OH and 3'-OH, and 4'-OH sites of the molecule NaQSA-8. Thermal decomposition of the compounds was carried out in air and nitrogen gas carries. The TG-FTIR-MS technique was employed to study decomposition pathway. The dehydration process was discussed in relation to of the nature of water contained in the compounds.

**Keywords** Sulfonic derivatives of quercetin · Spectroscopic properties · Thermal analysis, TG-FTIR-MS

## Introduction

Flavonoids form a widely distributed class of naturally occurring pigments present in vascular plants and are responsible for much of the coloring in nature. They occur in fruits, vegetables, nuts, seeds, stems, and flowers as well

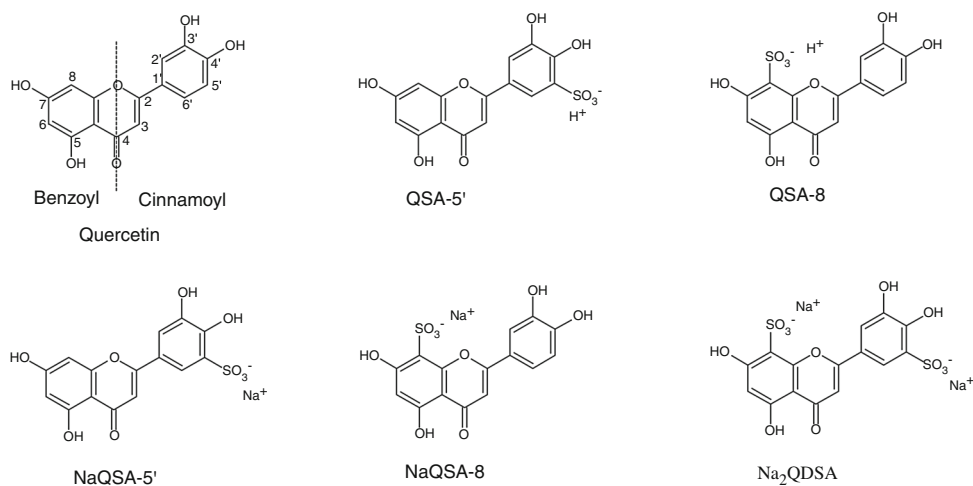
as tea, wine, propolis, and honey. Flavonoids exhibit a broad spectrum of biological activities: cardiovascular protection [1], antioxidant [2], anticancer [3], antiviral [4], anti-inflammatory, and antimicrobial activity [5]. They have played major roles in successful medical treatments in ancient as well as modern times [6].

In addition to their biological activities, flavonoids also have the ability to chelate metals ions. This metal chelation enhances the biological behavior of the flavonoids. Most of the studies of metal complexation were done in non-aqueous solvents or mixed solvent systems due to the limited solubility of flavonoids in purely aqueous solution. Much better solubility is shown by some sulfonic derivatives of flavonoids and, at the same time, they retain the properties of the parent compounds, Fig. 1. Moreover, in the presence of strongly acidic sulfonic group in the molecule the range of complexation of metal ions becomes markedly larger compared with parent flavonoids. The sulfonic derivatives of quercetin form complexes with metals at low pH (pH about 2), whereas quercetin at pH of about 5 [7, 8]. The introduction of sulfonic group to the quercetin, increases the ligand's acidity and precludes the metal ions from hydrolysis [7]. The derivatives of quercetin are not selective reagents. However, they have relatively high absorptivities of  $\sim 10^4$  L mol $^{-1}$  cm $^{-1}$ . These properties and their good solubility in water render them as promising analytical reagents for a metal ion determination. In the papers [9, 10], the QSA and sodium salt moryno-5'-sulfonic acid (NaMSA) were proposed as analytical reagents for the spectrophotometric determination of metals.

Furthermore, NaQSA and NaMSA, due to the ability to chelate metals ions, have been applied in the studies on detoxification of mercury, cadmium, and lead in rats [11–13]. It is worth emphasizing that NaQSA and NaMSA

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**Fig. 1** Structure of quercetin and its sulfonic derivatives



exert low toxicity. Therefore, they are supposed to be useful for a therapy.

In the present work, we focused on new sulfonic derivatives of quercetin as quercetin-8-sulfonic acid (QSA-8) and its sodium salt (NaQSA-8). They are of interest for their potential anti-inflammatory and antiviral properties. NaQSA-8 compound is an excellent antagonist of *Vif* which has been found to be a novel hit of *HIV-1* [14]. The synthesis of NaQSA-8 was already described, and the structure of the compound was determined by the X-ray analysis [15]. However, spectroscopic studies and studies of speciation in solution of the ligand were not carried out. So, in the first step of this work, we improved the conditions of synthesis and NaQSA-8 and developed a method of obtaining of quercetin-8-sulfonic acid. Then, the study of speciation of both ligands in a solution was performed. The molecular properties and structure of the compounds were investigated by thermogravimetric analyses and spectral methods.

In order to confirm complexing properties of quercetin sulfonic derivatives, complex of Fe(II) ions with NaQSA-8 in the solid state was synthesized. Fe(II) ions have been chosen for these studies due to the fact that Fe(II) is one of the most prevalent transition metals, which is essential for proper development of plants, animals, and humans.

## Experimental

### Materials

Quercetin (Chemapol), concentrated sulfuric acid ( $d = 1.84 \text{ g cm}^{-3}$ ), sodium chlorate(VII), sodium hydroxide, hydrochloric acid, chloric acid(VII), ammonium iron(II) sulfate hexahydrate, were used for the experiments. All the reagents were analytically pure.

### Instrumentation and measurement

Elemental analysis for C, H, N, and S was performed with an Elemental Analyzer EA 1108 apparatus (Carbo Erba, Italy). The contents of the sodium and iron, respectively, were determined by an AAS Perkin Elmer 3100 spectrophotometer (Perkin Elmer, USA). Anal. Calcd for  $\text{C}_{15}\text{H}_{10}\text{O}_{10}\text{S}\cdot 4\text{H}_2\text{O}$ : C, 39.64; H, 3.99; S, 7.05;  $\text{H}_2\text{O}$ , 15.86. Found: C, 40.56; H, 3.65; S, 7.58;  $\text{H}_2\text{O}$ , 16.00. Anal. Calcd for  $\text{C}_{15}\text{H}_9\text{O}_{10}\text{SNa}\cdot 4\text{H}_2\text{O}$ : C, 37.81; H, 3.61; S, 6.73; Na, 4.82;  $\text{H}_2\text{O}$ , 15.12. Found: C, 37.63; H, 3.18; S, 6.36; Na, 4.15;  $\text{H}_2\text{O}$ , 15.10. Anal. Calcd for  $\text{Fe}_3(\text{C}_{15}\text{H}_7\text{O}_{10}\text{SNa})_2(\text{OH})_2\cdot 12\text{H}_2\text{O}$ : C, 29.47; H, 3.29; S, 5.24; Na, 3.76; Fe, 13.70;  $\text{H}_2\text{O}$ , 17.68. Found: C, 29.23; H, 2.85; S, 6.17; Na, 4.21; Fe, 13.70;  $\text{H}_2\text{O}$ , 18.34. No nitrogen was assayed in the compounds. The potentiometric titration was performed with a N5172 pH-meter with glass electrode. The protonation constants of QSA-8 and NaQSA-8 were determined by spectrophotometric and potentiometric methods at  $25 \pm 1 \text{ }^\circ\text{C}$  for  $I = 0.1$  ( $\text{NaClO}_4$ ) with the N5172 pH-meter with glass electrode. The initial concentration of the solution sulfonic derivatives was determined spectrophotometrically and potentiometrically by the Gran method [16]. The glass electrode was calibrated for pH determination by generating a calibration curve of calculated  $E = f(\text{pH})$  from known amounts of added acid or base in  $0.1 \text{ mol dm}^{-3}$   $\text{NaClO}_4$  solution. The series of measurements were carried out at three various concentrations  $2 \times 10^{-3}$ ,  $1 \times 10^{-3}$ , and  $4 \times 10^{-3} \text{ mol dm}^{-3}$ . These solutions were titrated with a  $0.1 \text{ mol dm}^{-3}$  sodium hydroxide solution. Potentiometric titration of the aqueous solutions of the tested compounds was carried out from  $2 \times 10^{-3}$  to  $4 \times 10^{-4} \text{ mol dm}^{-3}$  concentration range.  $3 \text{ cm}^3$  of  $0.1 \text{ mol dm}^{-3}$  HCl solution was added to the titrated NaQSA solutions. The constant ionic strength of the solutions was maintained at 0.1 with using  $2 \text{ mol dm}^{-3}$   $\text{NaClO}_4$ . During the titration, the solutions were thermostated at  $25 \pm 1 \text{ }^\circ\text{C}$  and stirred continuously. The

**Table 1** Protonation constants of QSA-8 and NaQSA-8 at  $25 \pm 1$  °C, at constant ionic strength  $I = 0,1$  (NaClO<sub>4</sub>)

Compound	Method	$\log K_1^H$	$\log K_2^H$	$\log K_3^H$	$\log K_4^H$	$\log K_5^H$
QSA-8	Potentiometric	–	10,26	9,18	8,09	–
	Spectrophotometric	–	–	–	–	7,97
NaQSA-8	Potentiometric	–	10,82	9,91	9,13	7,96
	Spectrophotometric	–	–	–	–	7,68

**Table 2** Thermal decomposition of QSA-8, NaQSA-8 and of complex NaQSA-8 with Fe(II) in air

Temperature interval/°C	$T_{min}^{DTG}/^{\circ}C$	$T_k/^{\circ}C$	$\Delta m/\%$	
			Calcd.	Obtain.
<b>C<sub>15</sub>H<sub>9</sub>O<sub>10</sub>S·4H<sub>2</sub>O</b>				
20–140	70.2		15.8 (H <sub>2</sub> O)	16.0
140–260	200.3			5.0
260–520	320.0			79.0
		>520	No residue	No residue
<b>C<sub>15</sub>H<sub>9</sub>O<sub>10</sub>SNa·4H<sub>2</sub>O</b>				
20–150	72.5		15.1 (H <sub>2</sub> O)	15.0
150–373	351.2			17.5
373–660	462.0			53.5
		>660	14.9 (Na <sub>2</sub> SO <sub>4</sub> )	14.0
<b>Fe<sub>3</sub>(C<sub>15</sub>H<sub>7</sub>O<sub>10</sub>SNa)<sub>2</sub>(OH)<sub>2</sub>·12H<sub>2</sub>O</b>				
20–160	78.8		17.7 (H <sub>2</sub> O)	18.3
160–313				9.3
313–500	411.3			38.7
500–650	591.8			0.5
		>650	32.1 (Na <sub>2</sub> SO <sub>4</sub> , Fe <sub>2</sub> O <sub>3</sub> )	33.2

$T_{min}^{DTG}$ —temperature corresponding to minimum on DTG curve

$T_k$ —temperature of final product formation

protonation constants were determined by the Rossatti method [17]. The results of these experiments are presented in Table 1 and Figs. 2 and 3. The thermogravimetric analysis was performed with a TGA/DSC1 apparatus (Mettler Toledo) between 20 and 800 °C in air and 25–1,000 °C in nitrogen at a heating rate 10 °C min<sup>-1</sup>, Fig. 4. For TG-FTIR-MS analyses, TG instrument was online coupled with FTIR apparatus Nicolet iS10 (Thermo Scientific) by a transfer line heated at 220 °C. The FTIR spectra of the evolved gases were recorded in the range of 400–4,000 cm<sup>-1</sup> with the resolution of 4 cm<sup>-1</sup>. The vapors from the gas cell were then transferred into MS spectrometer ThermoStar<sup>TM</sup> (Pfeiffer Vacuum) by quartz capillary, heated at 200 °C. The data were scanned in the range  $m/z = 1$ –100 with the scanning rate of 500 ms/amu. The results are listed in Tables 2 and 3. The differential scanning calorimetry (DSC) was performed with a Mettler Toledo DSC-822° calorimeter. The differential scanning calorimetry of NaQSA-8 and Fe(II)-NaQSA-8 was carried out in nitrogen atmosphere between 0 and 300 °C, at a heating

**Table 3** Thermal decomposition of NaQSA-8 and of complex NaQSA-8 with Fe(II) in nitrogen

Temperature interval/°C	$T_{min}^{DTG}/^{\circ}C$	$T_k/^{\circ}C$	$\Delta m/\%$	
			Calcd.	Obtain.
<b>C<sub>15</sub>H<sub>9</sub>O<sub>10</sub>SNa·4H<sub>2</sub>O</b>				
25–161	66.3		9.4 (H <sub>2</sub> O)	9.0
161–233	176.7		1.9 (H <sub>2</sub> O)	1.5
233–413	352.9			28.0
413–697	475.6			15.5
697–885	764.4			19.7
		>885		26.3
<b>Fe<sub>3</sub>(C<sub>15</sub>H<sub>7</sub>O<sub>10</sub>SNa)<sub>2</sub>(OH)<sub>2</sub>·12H<sub>2</sub>O</b>				
25–157	75.3		13.3 (H <sub>2</sub> O)	13.4
157–273	230.3			11.4
273–397	328.5			11.7
397–489	455.6			8.0
489–609	538.7			10.2
609–860	726.3			18.3
		>860		27.0

$T_{min}^{DTG}$ —temperature corresponding to minimum on DTG curve

$T_k$ —temperature of final product formation

rate 10 °C min<sup>-1</sup> for a sample mass 10,63 mg, Fig. 9. The UV–Vis spectra of the compounds in water were taken with a Beckman DU-640 spectrophotometer (Beckman, Germany), Fig. 10. Infrared spectra were carried out in KBr pellets in 4,000–500 cm<sup>-1</sup> on an FTIR Paragon 100 spectrophotometer (Perkin Elmer, USA). The results for all studied compounds are collected in Table 4. The <sup>1</sup>H NMR spectra in DMSO-*d*<sub>6</sub> were recorded with a BF 587A TESLA FT NMR 80 MHz instrument. The results obtained are listed in Table 5.

#### Synthesis of quercetin-8-sulfonic acid (QSA-8) and its sodium salt (NaQSA-8)

8 cm<sup>3</sup> of concentrated sulfuric acid ( $d = 1.84$  g cm<sup>-3</sup>) was added to 2 g of quercetin in a 100 cm<sup>3</sup> round-bottom flask. The reaction mixture was vigorously stirred for 2 h at 18–20 °C. Then, 20 cm<sup>3</sup> of very cold water was added into the reaction mixture. The orange–red precipitate was filtered at a reduced pressure and recrystallized twice from the hot saturated water solution. Next, the orange–red

**Table 4** UV–Vis and IR spectra sulfonic derivatives of quercetin and complex with Fe(II)

Quercetin (own research)	C <sub>15</sub> H <sub>9</sub> O <sub>10</sub> S·4H <sub>2</sub> O	C <sub>15</sub> H <sub>9</sub> O <sub>10</sub> SNa·4H <sub>2</sub> O	Fe <sub>3</sub> (C <sub>15</sub> H <sub>7</sub> O <sub>10</sub> SNa) <sub>2</sub> (OH) <sub>2</sub> ·12H <sub>2</sub> O	
UV–Vis $\lambda$ nm ( $\epsilon \times 10^4$ L mol <sup>-1</sup> cm <sup>-1</sup> )				
372 (2.40)	367 (2.19)	366–367 (2.28)	430	Band I
256 (2.69)	254 (1.74)	254 (1.80)	268	Band II
IR frequencies/cm <sup>-1</sup>				
3,600–2,200	3,600–2,100	3,600–2,000	3,600–1,900	Tentative assignment
(3,400, 3,309, 2,983, 2,805, 2,719)	(3,290, 3,126, 2,765, 2,608)	(3,363, 3,230, 2,826)	(3,187)	$\nu(\text{O–H})$ , $\nu(\text{C–H})$
1,664	1,634	1,656	1,633	$\nu(\text{C=O})$
1,612	1,600	1,604	1,593	$\nu(\text{C=C})+$
1,560	1,578	1,558	1,570	$\nu(\text{C=O})$
1,521	1,541	1,525	1,517	
1,513		1,504		
1,462	1,442	1,461	1,479	$\nu(\text{C–O})$
1,434	1,420	1,445		$\delta(\text{O–H})$ , $\nu(\text{C–O})$
1,380	1,390	1,383	1,402 1,364	
1,260	1,274	1,269	1,271	$\nu(\text{C–C})$ , $\nu(\text{C–C–O–C})$
1,243	1,234	1,247		
	1,199	1,192	1,195	$\nu_{\text{asym}}(\text{SO}_2)$
	1,159	1,160	1,158	
1,014	1,022	1,014	1,020	$\delta(\text{O–H})$
960–620	960–620	960–620	960–620	$\gamma(\Phi)(\text{C–H})$
			520	$\nu(\text{M–O})$

Symbols  $\nu$  stretching mode,  $\delta$  in plane deformation,  $\gamma$  out of deformation,  $\Phi$  aromatic ring

**Table 5** The  $\delta$  (ppm) values of the aromatic protons of quercetin and its sulfonic derivatives in DMSO-*d*<sub>6</sub>

Proton	Quercetin [26]	Quercetin	QSA-8	NaQSA-8
H-6	6,28	6,15	6,12	6,09
H-8	6,52	6,34	–	–
H-5'	7,01	6,88	6,89	6,86
H-6'	7,61	7,45	7,84	7,80
H-2'	7,78	7,61	7,97	7,94

sediment was dried in air at room temperature. The synthesis yield was 40 %.

The sodium salt (NaQSA-8) was obtained by neutralizing the post-sulfonic mixture with a 20 % solution of NaOH up to pH 4. The precipitated yellow sediment was filtered off and crystallized twice from the saturated water hot solution. The yellow sediment was dried in air at room temperature. The synthesis yield was 44 %.

Synthesis of the Fe<sup>2+</sup> complex with the sodium salt of quercetin-8-sulfonic acid

The iron complex was obtained by mixing 0.01 mol dm<sup>-3</sup> aqueous solution of Fe(II) (ammonium iron(II) sulfate,

hexahydrate) with 0.002 mol dm<sup>-3</sup> aqueous solution of NaQSA-8, in the molar concentrations ratio  $c_M:c_L = 7:1$  ( $c_M$ —molar concentration of Fe<sup>2+</sup>,  $c_L$ —molar concentration of NaQSA-8). At the beginning 0.0762 g of NaQSA-8 (C<sub>15</sub>H<sub>9</sub>O<sub>10</sub>SNa·4H<sub>2</sub>O) was placed in a beaker, and then 80 cm<sup>3</sup> of distilled water was added. The mixture was heated until the ligand underwent complete dissolution, and then 112 cm<sup>3</sup> of Fe<sup>2+</sup> solution was added. A yellow–green solution was obtained after the substrates mixing. After cooling to room temperature, the mixture was neutralized to pH 5 by means of 0.1 and 0.01 mol dm<sup>-3</sup> NaOH solution. After a few minutes, sediment precipitated which, after 24 h, was filtered off and rinsed several times in redistilled water. Obtained black sediment was dried in air and the dark at room temperature. The synthesis yield was 46 %.

## Results and discussion

Depending on the conditions of quercetin sulfonation, the sulfonic group can occur in location C<sub>5'</sub> (QSA-5') or C<sub>8</sub> (Na<sub>2</sub>QSA). This paper describes the synthesis conditions where the sulfonic group occurs in position C<sub>8</sub>.

They are as follows:  $\text{H}_2\text{SO}_4$  concentration of  $d = 1.84 \text{ g cm}^{-3}$ , temperature 18–20 °C, sulfonation time 2 h. The composition and the structure of the sulfonic derivatives of quercetin (QSA-8 and NaQSA-8) were determined by the elemental analysis, thermogravimetric methods, UV–Vis, IR, and  $^1\text{H}$  NMR spectra. The molecular formula of an orange–red sulfonic acid is the  $\text{C}_{15}\text{H}_{10}\text{O}_{10}\text{S}\cdot 4\text{H}_2\text{O}$  and of its yellow salt is  $\text{C}_{15}\text{H}_9\text{O}_{10}\text{SNa}\cdot 4\text{H}_2\text{O}$ . The solubility of the compounds in water is of the order  $\cdot 10^{-3} \text{ mol dm}^{-3}$ . Synthesis of the complex of NaQSA-8 ( $\text{C}_{15}\text{H}_9\text{O}_{10}\text{SNa}\cdot 4\text{H}_2\text{O}$ ) with Fe(II) ions was carried out at pH 5 and with excess of the metal ions molar concentration in relation to the ligand ( $c_{\text{M}}:c_{\text{L}} = 7:1$ ). The black complex with yield 46 % was obtained.

The composition of the  $\text{Fe}_3(\text{C}_{15}\text{H}_7\text{O}_{10}\text{SNa})_2(\text{OH})_2\cdot 12\text{H}_2\text{O}$  complex was identified using elemental analysis, thermogravimetric analysis, differential scanning calorimetry, electronic spectra, and infrared spectra. The obtained results show that this compound is highly hydrated and contains 12 water molecules. It is insoluble in water and very slightly soluble in methanol.

The acid–base properties of the compounds (dissociation and protonation constants) were determined by potentiometric and spectrophotometric methods. Quercetin-8-sulfonic acid ( $\text{H}_5\text{A}^-$ ) possesses five hydroxyl groups which undergo dissociation with an increase of pH. To examine the equilibria of metal ion complexation, it is necessary to know the values of ligand protonation constants. Their acid–base equilibria are crucial for anticipating the quantitative interrelations of forms with different protonation degrees in physiological liquids of specific pH values [18].

The stepwise protonation constants (Table 1) were calculated based on the general formation constants. The spectrophotometric method was used for finding only the first dissociation constant of QSA-8 and NaQSA-8 within the 6–9 pH range. The dissociation constant was determined based on the Babko method [19].

The obtained values for QSA-8 and NaQSA-8 are  $1.08 \times 10^{-8}$  (7.97) and  $2.1 \times 10^{-8}$  (7.68), respectively. Figures 2 and 3 give a list of the mole fractions of the particular ion forms with regard to pH. The values of the formation constants for potentiometric method are 9.84, 19.02, and 29.28. For NaQSA-8, the formation constants values are 7.96, 17.09, 27.00, and 37.83. The values for QSA-8 and for QSA and NaQSA-8 were not obtained from experimental data. The protonation constant values presented in Table 1 result by mathematical calculation from the general formation constants. The value was not found because in sulfonic derivative solutions at  $\text{pH} > 10$  side reactions occur.

Thermal analysis by TG–DTA technique was proved to be very useful in determining the kind of water content in

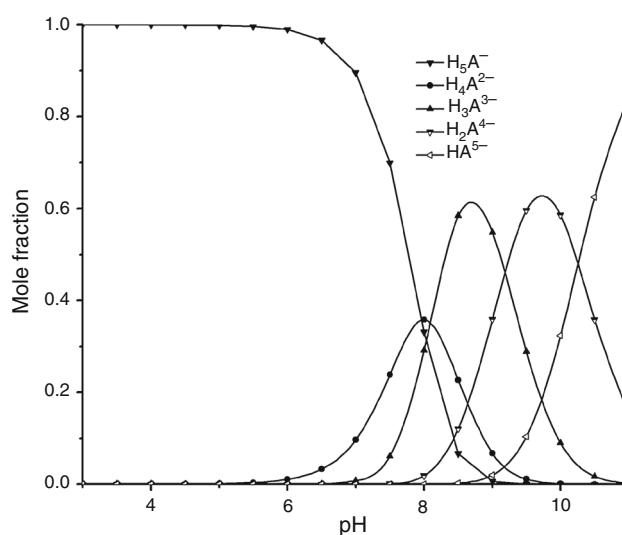


Fig. 2 Species distribution of QSA-8 as a function of pH

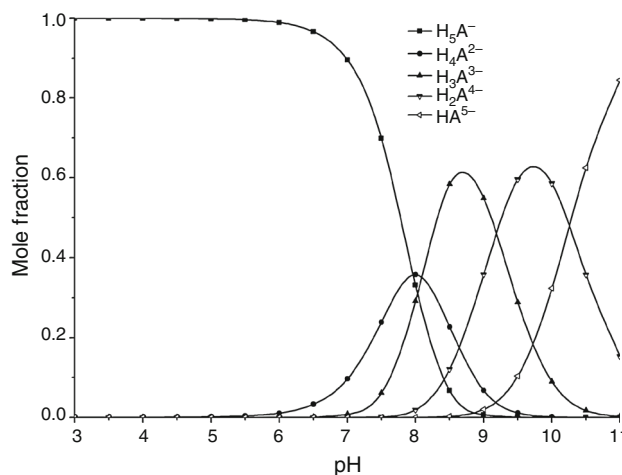
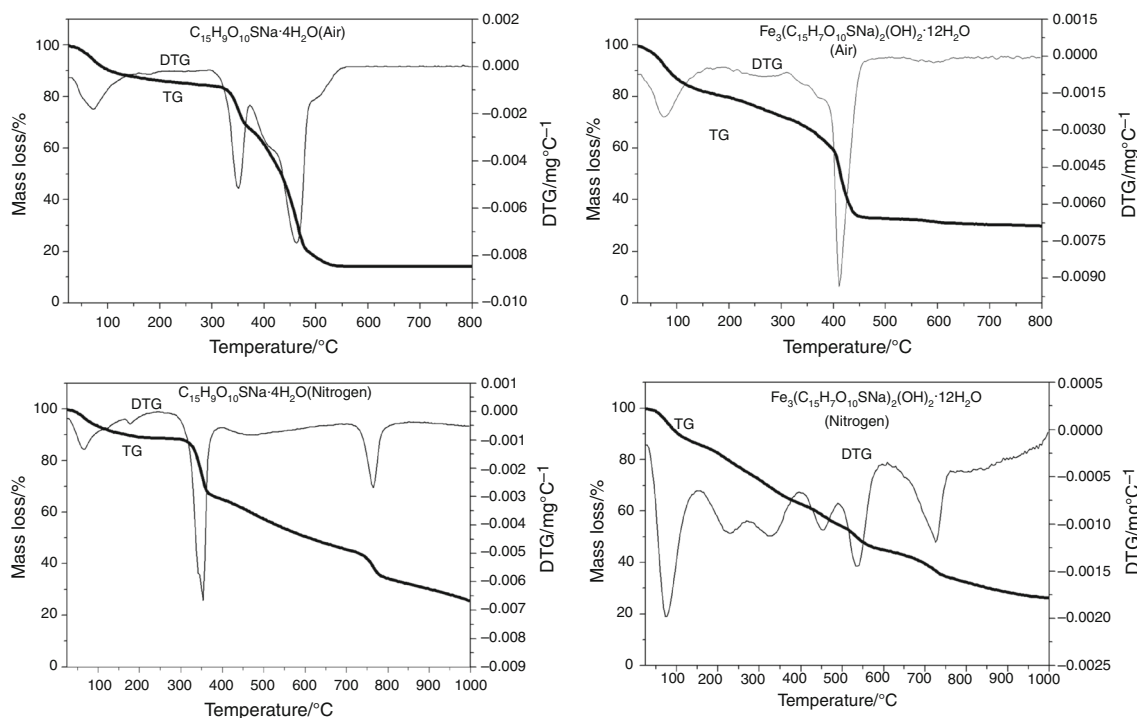


Fig. 3 Species distribution of NaQSA-8 as a function of pH

the compounds and their thermal stability and decomposition mode under controlled heating rate. The thermal studies were performed in air and nitrogen atmosphere, Fig. 4. The course of the decomposition of the compounds is different in both gas carriers. In the first step, the thermal decomposition of the compounds was studied in air within 20–800 °C (Table 2). The obtained results provide the direct evidence that the studied compounds undergo multistep changes. The first one is related to dehydration process, whereas the next stages are related with degradation of compounds. In the thermogravimetric analysis of the compounds, the one step of dehydration is observed and related to the endothermic effect on the DTA curve in following temperature range 20–140 °C for QSA-8, 20–150 °C for NaQSA-8 (loss of 4 water molecule), and 20–160 °C for the complex (12 molecules of water) [20].



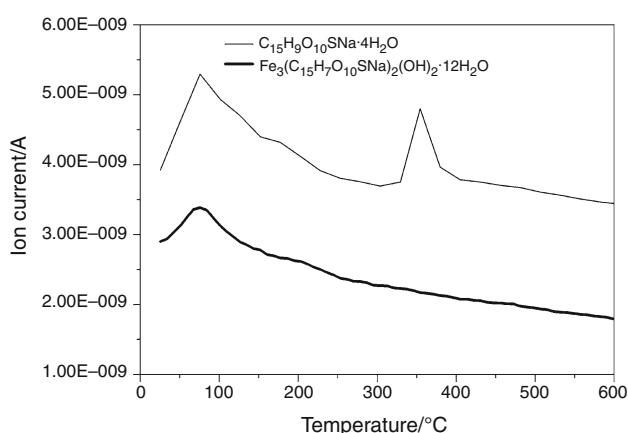
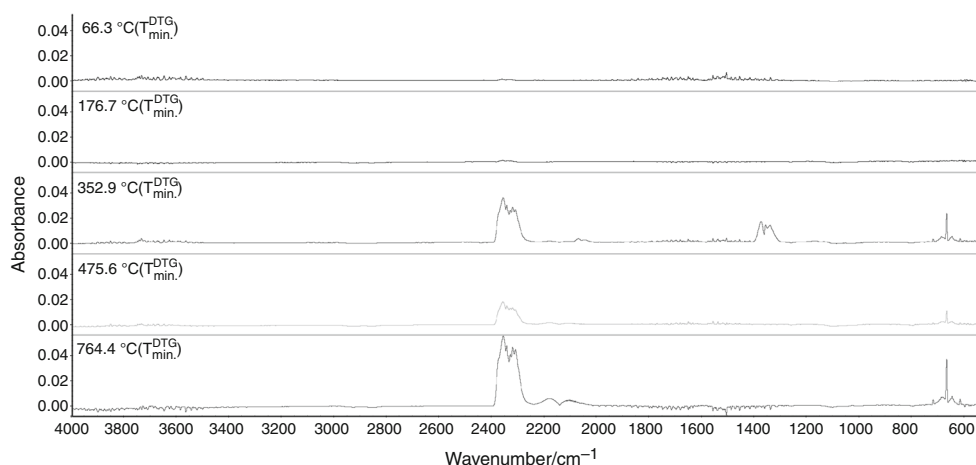
**Fig. 4** TG/DTG curves of NaQSA-8 and Fe(II) complex with NaQSA-8 in air and nitrogen atmosphere

The further increase of temperature causes processes of decomposition of anhydrous compounds. This stage is accompanied by a complex exothermic effect on the DTA curve in the temperature range 150–560 °C, suggesting that rather oxidation of the sample takes place along with the decomposition. Above 650 °C (NaQSA-8 and of complex with Fe(II)), the final decompositions products, oxides, and salts of the appropriate metal were formed. For QSA-8 the final decompositions product has not been formed, since this compound above 520 °C was decomposed completely (100 % mass loss), Table 2.

The thermal degradation of NaQSA-8 and the complex with Fe(II) has also been recorded in nitrogen atmosphere with FTIR analysis of gaseous products of decomposition and the coupled TG-mass spectrometry (MS). The comparison of the TG data for NaQSA-8 in nitrogen and air atmosphere, reveals that the substance shows different thermal behaviors, Fig. 4. Thermal analysis of NaQSA-8 in nitrogen indicates that it undergoes five-step decomposition (Table 3), accompanied by two endothermic and three exothermic effects on DTA curves. It seems that the dehydration process of NaQSA-8 occurs in two steps. The first step at range 25–165 °C is connected with loss of 2.5 water molecules, whereas the next dehydration takes place at about 220 °C and is connected with loss of 0.5 of water molecule. This process is accompanied by weak endothermic peak at  $T_{\max}$  177 °C. The FTIR and MS spectra of gaseous products of decomposition of NaQSA-8 clearly confirm that the first

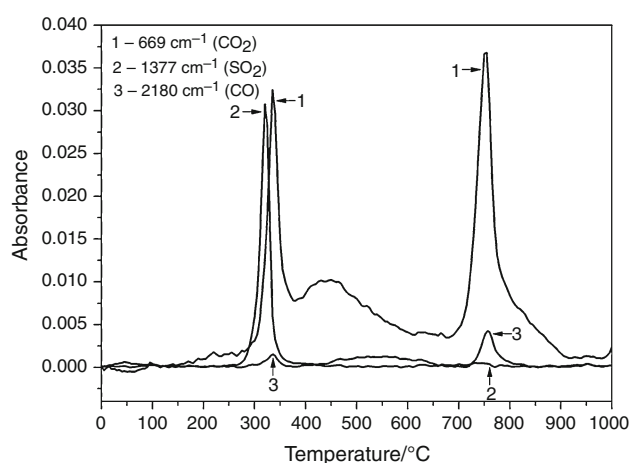
and second stages of decomposition of the compounds are connected with dehydration process. The FTIR spectra (Fig. 5) show bands in the wavenumber ranges 3,700–3,600  $\text{cm}^{-1}$  and 1,600–1,500  $\text{cm}^{-1}$ , corresponding to stretching and deformation vibrations of water molecules, respectively. Furthermore, the ion peak with  $m/z$  values 18 ( $\text{H}_2\text{O}^+$ ) in the MS spectra confirms this assumption. It is noteworthy that based on MS spectra dehydration is continued at higher temperatures over 320 °C, resulting a higher ion current, Fig. 6 [21]. It corresponds to release of the next water molecule. Stepwise dehydration process illustrates different modes of water molecules bonding. It can be concluded that the first stage of NaQSA-8 dehydration corresponds to release of weakly bonded water molecules. Probably, these molecules are connected with flavonoid ligand via the hydrogen bonds. The water molecules removed in the second step of dehydration are more tightly bonded, and they are released in significantly higher temperature. It is assumed that these water molecules are probably directly bonded with metal atoms [22]. This can be considered as expected result according to the crystal structure of NaQSA-8 presented in [15] where two water molecules are coordinated to sodium cation, and one is crystalline water. Moreover, the water molecules take part in inter- and intramolecular bonds with different interatomic distances. These differences are also visible in dehydration steps [23]. The third step of decomposition is not a single one but overlapping of several processes according to the DTA

**Fig. 5** The FTIR spectra in nitrogen of gaseous species produced during decomposition of NaQSA-8 at different temperatures



**Fig. 6** MID curves of NaQSA-8 and Fe(II) complex with NaQSA-8

curve profile. Exothermic peaks observed in the 290–395 °C temperature range are probably due to both endothermic and exothermic reactions that occur simultaneously. These decomposition processes generate H<sub>2</sub>O, SO<sub>2</sub>, CO<sub>2</sub>, and CO in the evolved gaseous products [24]. The FTIR spectra show characteristic absorption bands at 2,358, 2,320 cm<sup>-1</sup>, and 669 cm<sup>-1</sup> related to stretching and deformations vibrations of carbon dioxide. Apart from that there also appear the bands at 1,340, 1,375 cm<sup>-1</sup>, and 1,137, 1,171 cm<sup>-1</sup>. These bands can be attributed to vibrations of SO<sub>2</sub>. It is noteworthy that SO<sub>2</sub> is evolved only in the third step of decomposition. Based on the analysis of the profile of changes of absorbance as a function of temperature recorded for SO<sub>2</sub> (1,375 cm<sup>-1</sup>), CO<sub>2</sub> (669 cm<sup>-1</sup>), and CO (2,180 cm<sup>-1</sup>) can be seen that the maximum concentration of sulfur dioxide is attained at a lower temperature than for the other ingredients of the evolved gas products, Fig. 7. This fact explains the appearance of conjugated peaks observed on the DTG curve in the third stage of degradation. In the last stage of decomposition apart from carbon dioxide, small amount of carbon monoxide can be observed. Vibrations of CO molecules give bands

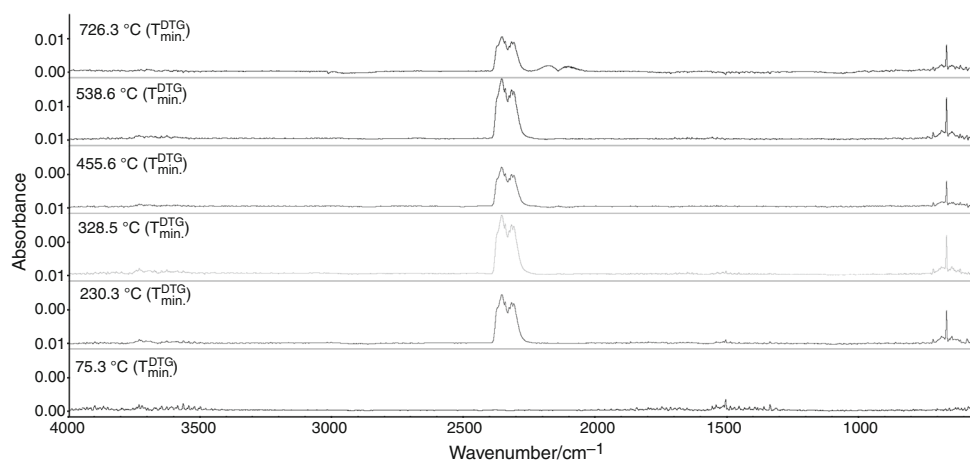


**Fig. 7** The profile of changes of absorbance as a function of temperature recorded for SO<sub>2</sub>, CO<sub>2</sub>, and CO during decomposition of NaQSA-8

at 2,180 and 2,105 cm<sup>-1</sup>. In the range 2,800–3,100 cm<sup>-1</sup> there are no absorption peaks, which may suggest that organic products are not formed as a result of decomposition of the sample.

The TG and DTG curves registered for complex of NaQSA-8 with Fe(II) are shown in Fig. 4 and indicate that the complex undergoes six steps thermal decomposition. The first step in the 25–157 °C temperature range, reflected by an endothermic effect, is connected with the elimination of water molecules. The ion peak with *m/z* 18 in the MS spectra confirms the presence of H<sub>2</sub>O in the evolved gaseous products in this step. The FTIR spectra also confirm that the first step of complex decomposition is dehydration process, as evidenced by the presence of bands of stretching vibrations of the OH group in H<sub>2</sub>O in the range 3,600–3,700 cm<sup>-1</sup> and the deformation vibrations in the range 1,500–1,600 cm<sup>-1</sup>. The temperature range of the first step of dehydration of the complex is comparable to that observed for NaQSA-8. However, the analysis of the FTIR

**Fig. 8** The FTIR spectra in nitrogen of gaseous species produced during decomposition of Fe(II) complex with NaQSA-8 at different temperatures



and MS spectra and recorded up to 157 °C suggests that water contained in the complex is released within a wider range of temperature, Fig. 8. In the FTIR spectrum, the bands of lower intensity corresponding to vibration of water molecules are presented. In the MS spectrum, the maximum evolution of H<sub>2</sub>O is observed in the 25–157 °C temperature range; when the temperature rises, some amount of water is removed, Fig. 6. However, the latter effect is not well separated, the thermal patterns do not clearly show the number of the water molecules. In order to determine the nature of water contained in the studied compounds, DSC analysis in nitrogen atmosphere was also performed. The DSC curves are shown in Fig. 9. The following enthalpy values per one molecule in the compounds were obtained: 55.3 kJ for NaQSA-8 and 52.6 kJ for the complex with Fe(II). As it can be seen, the enthalpy values are closed, which is the evidence of the similar force of binding water molecules in the ligand and its complex [25]. The obtained results of the thermal analysis suggest that part of water molecules present in the complex is hydrogen-bonded to the first-sphere ligands, whereas remaining ones, presumably are coordinated to metal atoms and are released within a broad range of temperature. The next stages of thermal change of the complex are due to the degradation of the ligand and reflected by endothermic peaks on the DTA curve. From the analysis of the FTIR spectra, it can be concluded that the major gaseous product of the decomposition of the complex is carbon dioxide, as confirmed by the presence of sharp bands of characteristic stretching vibrations of C–O group at 2,358 and 2,320 cm<sup>-1</sup>, and deformation vibrations at 669 cm<sup>-1</sup>. The absorbance of these bands is different for each stage of decomposition and shows the highest value at the fifth stage of degradation and the lowest at stage six. In the last step of decomposition in the gaseous products except the carbon dioxide, the carbon monoxide is also presented, as demonstrated by the characteristic bands at

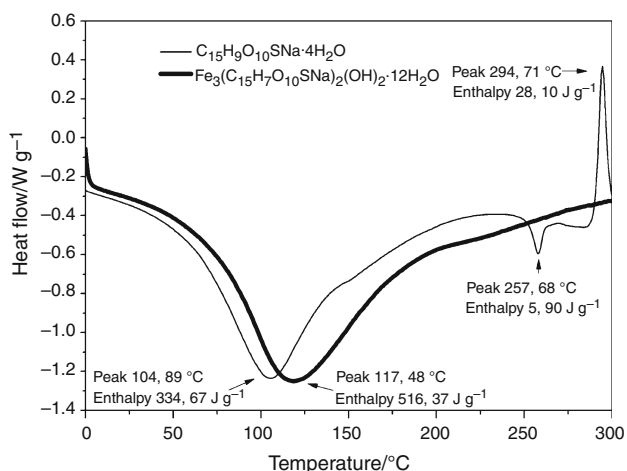
2,180 and 2,105 cm<sup>-1</sup>. The absence of any band in the range of 1,300–1,400 cm<sup>-1</sup>, and 110–1,200 cm<sup>-1</sup> in the spectra of the complex with Fe(II) indicates that sulfur dioxide is not formed as a result of decomposition of the compound. It can therefore be assumed that the non-volatile sulfur compounds are formed, which are present in solid products of degradation.

The UV–Vis spectra of the investigated compounds (QSA-8 and NaQSA-8) were taken in water (Fig. 3) and characterized by two bands: band I of  $\lambda(I)_{\max} = 367$  nm for QSA-8 and 366–367 nm for NaQSA-8, and band II of  $\lambda(II)_{\max} = 254$  nm for QSA-8 and NaQSA-8. The band I is due to the transition localized within the B ring of cinnamoyl system (with the 3C–OH group), and the band II is consistent with the absorbance of the ring A of benzoyl system (with the 5C–OH group). They are related to the  $\pi\text{--}\pi^*$  transitions [26, 27] and have high absorption coefficients ( $\epsilon$ ), Table 3.

In the case of the Fe(II) complex with NaQSA-8 (Fig. 10), the bands I and II are bathochromically shifted by 63 and 14 nm, respectively (Table 4). The increased absorbance in the range 500–600 nm suggests that in this area there is a weak band, which is connected with d–d transition in the iron ion. This band is covered by the intense charge-transfer band (charge-transfer transition L → M). This red shift is caused by the increased conjugative effect when complex is formed to give a new ring. The observed large changes at band I position might be evidence for the metal binding with this part of the ligand. The chelating properties of flavonoids toward metal ions have been attributed to the presence of the 3-hydroxy and 4-carbonyl group in the C-ring [25].

The UV–Vis spectra give significant information about co-ordination sites. As the 3-hydroxy group position has a more acidic proton, therefore the 3-OH is the first site to be involved in the complexation process [28]. The hydroxyl group of 3' and 4' position can bind a second metal ion. The information strongly suggests that these positions form





**Fig. 9** DSC curves of NaQSA-8 and Fe(II) complex with NaQSA-8

the stable chelate rings (five-membered). Similar results were obtained for the complex of Al(III) with quercetin. According to Porter et. al. [29] quercetin as a ligand (and probably quercetin sulfonic derivatives, too) possesses three possible chelating sites, of which only the 3-OH and pyrocatechol sites (the 3'-OH and 4'-OH) may be expected to be involved in the complex formation when stoichiometry of the final compounds is  $Al_2L$ , Al—aluminum cation, L—quercetin molecule.

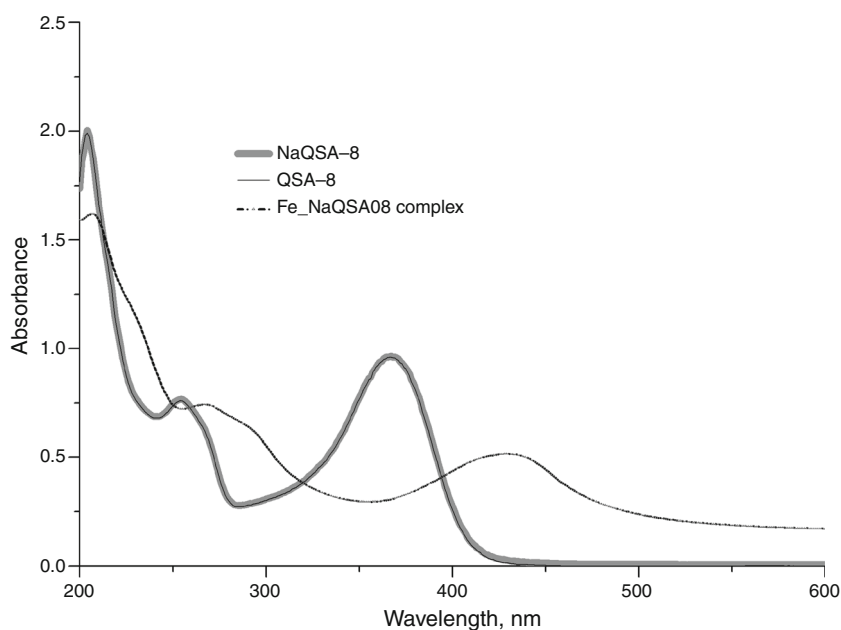
The IR spectra were recorded between 4,000 and  $500\text{ cm}^{-1}$ , in KBr pellets, and have been used to determine the compounds structure. The IR spectra of the compounds are similar and were interpreted on the basis of main bands. The tentative assignment is listed in Table 4. The infrared spectra of the studied compounds are characterized by a

broad band in the region  $3,600\text{--}3,000\text{ cm}^{-1}$  due to the presence of stretching vibrations  $\nu(\text{OH})$  of hydroxyl group in hydrogen bonded water molecules, which coincides with the results of the thermal analysis. The spectrum of QSA-8 shows band at  $1,634\text{ cm}^{-1}$  driving from vibrations of CO carbonyl group. The bathochromic shift of this band to lower frequencies in relation to the position of the band in the spectrum of quercetin ( $1,664\text{ cm}^{-1}$ ) is probably due to the intramolecular hydrogen bond formed with the strongly acidic hydrogen of the sulfonic group [30]. In contrary, the sodium cation in NaQSA-8 does not directly interact with the  $4\text{C}=\text{O}$  group. Hence, the frequency of the carbonyl group in the spectrum of NaQSA-8 is  $1,657\text{ cm}^{-1}$  and is closed to the value observed for quercetin ( $1,664\text{ cm}^{-1}$ ). In the spectra of the studied sulfonic derivatives, two frequencies were ascribed to the asymmetrical  $-\text{SO}_2$  vibrations:  $1,199, 1,159\text{ cm}^{-1}$  and  $1,192, 1,156\text{ cm}^{-1}$  for 8-QSA and NaQSA-8, respectively [31, 32].

The position of  $\nu(\text{C}=\text{O})$  is diagnostic for the involvement of the  $4\text{C}=\text{O}$  chromophore in the coordination. The complexation should lengthen the C—O bond in the carbonyl group and lessen the force constant, which, in turn, may shift the IR band of the carbonyl group toward a smaller wavenumber [33].

In the IR spectrum of the iron complex, the band of  $4\text{C}=\text{O}$  is shifted to a lower frequency in relation to the position of this band in NaQSA-8 ( $\Delta\nu = 24\text{ cm}^{-1}$ ). This placement suggests the coordination of the carbonyl group with the metal cation [34]. In addition, changes of intensity of bands at  $1,479, 1,364\text{ cm}^{-1}$  attributed to vibrations of C—OH and/or C—O<sup>-</sup> of the catechol groups of the B ring and were visible a new band appeared at  $1,402\text{ cm}^{-1}$ .

**Fig. 10** Absorption spectrum of sulfonic derivatives of quercetin and of complex NaQSA-8 with Fe(II) in water. Concentration of the solutions  $4.4 \times 10^{-5}\text{ mol L}^{-1}$ ; (Black rectangle) QSA-8,  $\lambda(\text{I})_{\text{max}} = 367\text{ nm}$ ,  $\lambda(\text{II})_{\text{max}} = 254\text{ nm}$ ; (Black rectangle) NaQSA-8,  $\lambda(\text{I})_{\text{max}} = 366\text{--}367\text{ nm}$ ,  $\lambda(\text{II})_{\text{max}} = 254\text{ nm}$ ; (Black rectangle White triangle Black rectangle) complex with Fe(II),  $\lambda(\text{I})_{\text{max}} = 430\text{ nm}$ ,  $\lambda(\text{II})_{\text{max}} = 268\text{ nm}$



These data support the conclusion that, second chelation site of Fe(II) ions takes place by the *ortho* hydroxyl groups in B ring [35]. On the other hand, the  $\nu(\text{C}-\text{C}-\text{O}-\text{C})$  and  $\nu(\text{C}-\text{C})$  frequencies of the ring changed slightly upon complexation, indicating that the ring oxygen does not form metal–oxygen bonds with metal ion [36]. The sulfonic group frequencies in Fe–NaQSA-8 spectra are similar to those in NaQSA-8. Slight shift in the position of these bands ( $\Delta\nu = 3 \text{ cm}^{-1}$ ) indicates that the sulfonic group does not participate in the metal bonding. In the spectra of the complex, there also appears a new band  $\nu = 520 \text{ cm}^{-1}$ , due to the formation of the M–O bond [37, 38].

The  $^1\text{H}$  NMR spectra of the obtained compounds were made to determine the substitution place of the sulfonic group in a quercetin molecule. The spectra were carried out in DMSO- $d_6$ . The  $\delta$  values of the aromatic protons of quercetin, and its sulfonic derivatives are listed in Table 5. Assignments for the aromatic protons of QSA-8 and NaQSA-8 were given by considering the available literature data and comparing it with the quercetin spectrum [39].

The lack of a signal corresponding to  $\text{C}_8$  proton in the  $^1\text{H}$  NMR spectra of the sulfonic derivatives shows that the substitution of sulfonic group in quercetin molecule takes place in the position occupied by the proton at  $\text{C}_8$ .  $^1\text{H}$  NMR spectrum of the Fe(II) complex was not carried out due to its slight solubility in DMSO- $d_6$ .

Quercetin and its sulfonic derivatives possess three possible chelating sites: the 3-hydroxy-4-keto, 5-hydroxy-4-keto, and 3',4'-ortho-dihydroxyl (catechol) groups [40]. In acidic solution, QSA forms complexes with the 4C=O and 3-OH groups as donor centers [41]. The complexation of quercetin with metal ions takes place at  $\text{pH} \geq 6$ , also with the involvement of the 4C=O and 3-OH. Other coordination centers (in neutral solutions, in the presence of an excess of metal ions) are the 4C=O and 5-OH, as well as the 3'-OH and 4'-OH. However, the most stable are the complexes with the 4C=O and 3-OH due to the formation of five-membered chelate rings [7, 42]. The authors of [43] suggest that for complex of quercetin containing one Fe(II) ion and molecule binding energy at the 4C=O and 3OH site is stronger than at the 3'-OH and 4'-OH site, while for complex containing two Fe atoms, bound at the 4C=O and 3-OH site and the 3'-OH and 4'-OH site is the optimal chelation site (have a preferred energy). It seems most likely that the chelation sites of NaQSA-8 with Fe(II) ions are the 4C=O, 3-OH and the 3'-OH, 4'-OH groups.

The obtained results of potentiometric and spectrophotometric investigations and literature data confirmed the spectroscopy studies. From the UV–Vis and IR spectra, it follows that the co-ordination sites of NaQSA-8 are localized as the hydroxyl groups of cinnamoyl system (the 3 and 3', 4' hydroxyl groups) and 4-carbonyl group. Taking

into account the synthesis environment ( $\text{pH} 5$ ), in the solution both  $\text{Fe}^{2+}$  and  $\text{Fe}(\text{OH})^+$  ions occur, so we assume that the  $\text{Fe}(\text{OH})^+$  ions are present in the complex [16].

## Conclusions

The sulfonic derivative of quercetin–quercetin-8-sulfonic acid (QSA-8) and its sodium salt (NaQSA-8) were formed during quercetin sulfonation by concentrated  $\text{H}_2\text{SO}_4$  in temperature 18–20 °C, by 2 h. In these conditions, the sulfonic group ( $-\text{SO}_3\text{H}$ ) substitutes in position  $\text{C}_8$  of the quercetin molecule. The sulfonic derivatives QSA-8 and NaQSA-8 are weak multiproton acids ( $\text{H}_5\text{A}^-$ ), are soluble in water, in which undergo stepwise dissociation in the range  $\text{pH} 6$ –10.5.

Sulfonic derivatives of quercetin form complexes with metal ions in aqueous solutions. The NaQSA-8 precipitates with Fe(II) ions solid complex at  $\text{pH} 5$  with the metal excess in relation to the ligand. The coordination sites of the metal are proposed as the 4C=O, 3-OH, and 3'-OH, 4'-OH groups of the molecule NaQSA-8. Thermal decomposition of the compounds was carried out in air, and nitrogen gas carries. The TG-FTIR-MS technique was employed to study decomposition pathway. The dehydration process was discussed in relation to of the nature of water contained in the compounds. The obtained results of the thermal analysis suggest that part of water molecules present in the compounds is hydrogen-bonded to the first-sphere ligands, whereas remaining ones, presumably are coordinated to metal atoms.

Sulfonic derivatives of quercetin are not selective reagents, but have relatively high absorptivities of  $\sim 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ . This property renders them as an analytical reagent for the spectrophotometric analysis of metals as well as in biology and medicine. The obtained new sulfonic derivatives of quercetin have not only cognitive but also practical importance as nontoxic compounds.

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