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Preparation and separation of mixed-ligand Fe(II) complexes containing 1,10-phenanthroline-5,6-dione as ligand

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The synthesis, separation, and characterization of mixed-ligand iron(II) complexes containing 1,10-phenanthroline (phen), 1,10-phenanthroline-5,6-dione (pdon), and NCS^- are reported. The mixed-ligand complexes $[\text{Fe}(\text{phen})(\text{pdon})_2]^{2+}$ and $[\text{Fe}(\text{phen})_2(\text{pdon})]^{2+}$ were prepared from iron(II) sulfate hepta hydrate and both ligands. The mixture of both complexes formed regardless the ratio of the ligands or the reaction time; therefore, the complexes were separated successfully on the reversed phase (RP) Develosil RP-Aqueous [C30] $5\ \mu\text{m}$, $150 \times 4.6\ \text{mm}$ column by two different methods. The first method was the ion paired RP chromatography performed under gradient elution with acetonitrile–water containing $0.001\ \text{mol L}^{-1}$ KPF_6 aqueous as mobile phases. The second method was the RP chromatography performed under gradient elution with methanol and water as mobile phases. The gradient elution with water–methanol as eluents was preferred for the semi preparative separations allowing one to use the complexes without further purification upon separation, different than the first method and its variations so far. Three complexes (**5**, **6**, and **7**) were characterized by electrospray ionization mass spectrometry, NMR, UV-Vis, and IR.

Keywords: Mixed-ligand iron(II) complexes; 1,10-Phenanthroline-5,6-dione; Synthesis; LC-MS

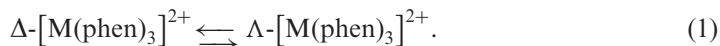
1. Introduction

Transition metal complexes with pyridyl ligands are very often seen in supramolecular chemistry. The selection of iron(II) is based on the fact that this ion is common in the living body and also due to its magnetic properties. 1,10-Phenanthroline (phen) and its derivatives, substituted at various positions (2,9 or 4,7, or 5,6 etc.), play an important role as ligands since they can be modified and used as a bridge between two metal centers, for either homo or heterometallic complexes [1, 2]. 1,10-Phenanthroline-5,6-dione (pdon) is of particular interest since two nucleophilic centers (nitrogen and oxygen lone pairs) are in a molecule similar to quinone, with all the atoms, except hydrogen atoms, being sp^2 hybridized [3]. The presence of two electronegative

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heteroatoms creates not only the basic properties in the Lewis sense but also, because of the resonance conjugation, makes it possible to alter the electron density in different parts of the molecule, especially by interaction of an external electrophile with unshared pairs of electrons of the heteroatom [4]. Therefore, pdon is a building block in binuclear or multinuclear complexes through reaction *via* a diiminic binding site and through an *o*-quinoid group [5].

Besides preparation of mono- and poly-nuclear metal complexes, increasing attention is given to the mixed-ligand transition metal complexes with these types of ligands [6]. This interest goes parallel to metal-DNA chemistry [7], catalysis [8], synthesis of building blocks for metallo-dendrimers [9], fabrication of high-performance materials [10], etc. Of course, we should not forget that mixed-ligand complexes are observed in biological systems or as the intermediates. Until recently, mixed-ligand complexes were limited to ruthenium complexes mostly due to their stability toward racemization, their intense metal-to-ligand charge transfer (MLCT) band in the visible spectrum, and their well-established preparation procedures [11]. Very little has been published about iron(II) mixed-ligand complexes due to difficulty in controlling their syntheses. In 1971, Taylor and Schilt [12] synthesized for the first time iron(II) mixed-ligand complexes with mixed α,α' -diimine complexes. He observed that some of the mixed complexes were not stable in solution. Mudasar *et al.* [13] reported the synthesis of iron(II) mixed-ligand complexes with phen and 4,7-diphenyl-1,10-phenanthroline. It is likely that the lack of the reports is due to instability of such complexes toward racemization, since it is known that mixed-ligand iron(II) complexes cannot be isolated in pure Λ or Δ form and that they easily racemize in solution (equation (1)).



Equation (1) indicates that there is a dynamic equilibrium from the Λ to Δ -enantiomer and *vice versa*.

The use of reversed phase (RP) HPLC for separation of organometallic and metal chelate complexes has been used for the past three decades [14]. Ion pair reversed chromatography is the chromatography of choice for separation of metal complexes when MS detection is not required.

Formation of solvent cluster ions occurs frequently due to association of solvent with cations and anions and thus results in complex electrospray ionization (ESI) mass spectra. The cluster ions could be eliminated by changing the MS conditions (de-clustering effect of an additional flow of a sheath gas or increasing the sampler skimmer cone voltage of the ESI interface), but those changes decrease the sensitivity of the detection. Elimination of cluster formation can be achieved by varying the composition of the mobile phase.

The aim of this work is to show that mixed-ligand iron(II) complexes with the ligands that are only slightly different can be prepared by their direct isolation from the mixture by RP HPLC without, so far, needed extraction when the separation was achieved by IP RP HPLC. The complexes were prepared from free ligands, 1,10-phenanthroline, and 1,10-phenanthroline-5,6-dione, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ stirring in water at room temperature; separated by HPLC coupled with UV-Vis or MS; and characterized by NMR, FT-IR, and electrospray ionization mass spectrometry (ESI-MS)/MS. The eventual goal is their use as synthons for stereospecific supramolecular synthesis.

2. Experimental

2.1. Chemicals and reagents

Solvents and reagents for the synthesis (as well as 1,10-phenanthroline and 1,10-phenanthroline-5,6-dione) were purchased from Aldrich; only NH_4NCS , NH_4PF_6 , and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were obtained from Kemika. Water (milli-Q, 18 m Ω) and other organic solvents for spectroscopic measurements were HPLC or spectroscopic grade purchased and used without purification. Filtration through 0.45 μm filters was performed for the HPLC use. There was no additional purification of chemicals performed.

2.2. Synthesis

$\text{Fe}(\text{phen})_2(\text{pdon})(\text{PF}_6)_2$ (**5**) and $\text{Fe}(\text{phen})(\text{pdon})_2(\text{PF}_6)_2$ (**6**). 1,10-Phenanthroline, 27 mg (1.5×10^{-4} mol), 1,10-phenanthroline-5,6-dione, 14 mg (6.66×10^{-5} mol), and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 22 mg (7.91×10^{-5} mol) were added to 3 mL of water and left to stir overnight at room temperature. 15.5 mg (1.43×10^{-4} mol) of NH_4PF_6 dissolved in 1 mL of water was added, black-brown precipitate formed was filtered off and washed with water and acetone, giving mixture of four products **3**, **5**, **6**, and **4**. The mixture was purified by analytical and semi-preparative HPLC in acetonitrile and aqueous 0.001 mol L $^{-1}$ KPF_6 as the mobile phases, yielding 15% of **3**, 22% of **5**, 15% of **6**, and 14% of **4**. The reaction mixture was also directly separated by HPLC in water/methanol solvent system. The counter ion for the separation with MeOH/water system was $(\text{SO}_4)^{2-}$ and there was no addition of NH_4PF_6 salt.

$\text{Fe}(\text{pdon})_2(\text{NCS})_2$ (**7**). 1,10-Phenanthroline-5,6-dione, 150 mg (7.14×10^{-4} mol) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 87 mg (3.1×10^{-4} mol) were added to 50 mL of water and left to stir for 2 h at room temperature. NH_4NCS , 60 mg dissolved in 6 mL of water was then added and left to stir 60 h. Black-purple precipitate formed was filtered off and washed with water and acetone, yielding 155 mg (84%) of pure product. IR (cm^{-1}): 3386, 3104, 3078, 3064, 2086, 2054, 1711, 1701, 1692, 1570, 1482, 1428, 1296, 1287, 1250, 1185, 1124, 1098, 1073, 1024, 936, 815, 807, 729, 720, 565. NMR ^1H (300 MHz, δ , DMSO, ppm): 8.62 (1H, s); 7.79 (1H, s); 7.64 (1H, s). ^{13}C (75.5 MHz, δ , DMSO, ppm): 174.48; 158.26; 129.96; 158.19; 136.5; 128.32. ESI-MS/MS in acetonitrile (m/z): 238.4 [$\text{Fe}(\text{pdon})_2$] $^{2+}$; 324.2 [$\text{Fe}(\text{pdon})(\text{NCS})$] $^{+}$; 364.7 [$\text{Fe}(\text{pdon})(\text{NCS})(\text{ACN})$] $^{+}$; 534.0 [$\text{Fe}(\text{pdon})_2(\text{NCS})$] $^{+}$.

The complexes $\text{Fe}(\text{phen})_3(\text{PF}_6)_2$ (**3**), $\text{Fe}(\text{pdon})_3(\text{PF}_6)_2$ (**4**) [15], and $\text{Fe}(\text{phen})_2(\text{NCS})_2$ (**8**) [16] were prepared according to previously described methods and used for characterization comparison.

2.3. Apparatus

HPLC: The analytical and semi-preparative separations were performed using the reverse phase Develosil RP-Aqueous [C30] 5 μm , 150 \times 4.6 mm column. The samples were prepared in the concentration range from 10^{-3} to 10^{-5} mol L $^{-1}$ in milli-Q water.

The first HPLC system consisted of Knauer HPLC system with two eluent module pumps K-501 with the inline solvent degasser, manual sample injector with 500 μL loop, and heated column department Knauer Jetstream Series with built-in Peltier thermostat with the operating range 5–85 $^\circ\text{C}$ (accuracy 0.1 $^\circ\text{C}$). The analyte detector module is

Wellchrom PDA K-2800 and the central control and the data output module were done by ChromGate software. The second HPLC system consisted of HP 1090 Diode Array HPLC system.

LC ESI-MS: The mass spectral data were acquired on an LCQ Deca ion trap mass spectrometer with Surveyor pump from Thermo Finnigan (San Jose, CA, USA) equipped with an ESI interface operated in positive ion mode. Nitrogen was used as auxiliary and sheath gas. Helium was used as collision gas in the ion trap.

Samples for direct injections were prepared in a concentration of about 0.05 mg mL^{-1} and directly injected by $250 \mu\text{L}$ Hamilton syringe pump at flow rate of about $5 \mu\text{L min}^{-1}$. MS spectra were recorded in ES positive ion mode in mass range 100–2000 amu. Sheath gas flow was set at 90 and auxiliary gas flow at 30 (arbitrary units). Spray voltage was set at 4.5 kV, while capillary temperature was 250°C and capillary voltage 17 V.

Samples for the chromatography separations were prepared in water and recorded in ES positive ion mode from 100 to 2000 amu. The reverse phase Develosil RP-Aqueous [C30] $5 \mu\text{m}$, $150 \times 4.6 \text{ mm}$ column was used at 25°C and the autosampler was set at 10°C . Sheath gas flow was set at 80 and auxiliary gas flow at 20 (arbitrary units). Source voltage was set at 5 kV, while capillary temperature was 350°C and capillary voltage 15 V. PDA detector collected the data from 190 nm to 600 nm and monitored at three wavelengths: (A) 254 nm, (B) 480 nm, and (C) 510 nm. A mobile-phase gradient was used at the flow rate $500 \mu\text{L min}^{-1}$ with methanol and water as mobile phase B. The mobile-phase gradient was used starting from linear gradient from 15% to 90% A in 45 min, followed by 90% A for 4 min, followed by a linear gradient from 50 to 60% A in 10 min.

IR: IR spectra of KBr pellets were recorded on a Bomem MB 102 spectrometer.

UV-Vis: UV-Vis spectra were collected by using a Varian Cary 50 spectrometer (1 cm quartz cell).

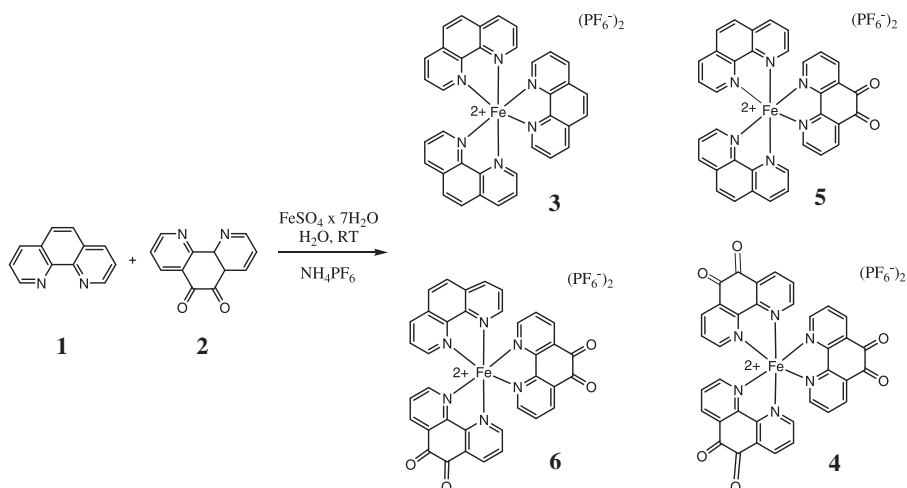
NMR: ^{13}C and ^1H spectra were recorded on a Bruker Avance 300 and Bruker Avance 600 spectrometer. The chemical shifts were expressed in ppm from TMS as determined with reference to the internal standard (DMSO and TMS).

3. Results and discussion

3.1. Synthesis of the iron(II) mixed-ligand complexes 5 and 6

We used one single procedure to prepare our iron(II) mixed-ligand complexes **5** and **6**. The iron(II) sulfate was mixed with both ligands at room temperature in water (scheme 1). Product composition could not be influenced by the ligand ratio or by the order of addition of components since the mixtures of the iron(II) complexes **3**, **4**, **5**, and **6** were always present.

There were attempts to perform selective recrystallization but the results were not satisfactory. We observed that the composition of the solution changed completely after a certain period of time. Basically, there were only two components left: brown precipitate (corresponding to **4**, and counter ion SO_4^{2-} , e.g. $\text{Fe}(\text{pdon})_3\text{SO}_4$) and red mother liquor (corresponding to **3**, and counter ion SO_4^{2-} , e.g. $\text{Fe}(\text{phen})_3\text{SO}_4$).



Scheme 1. Formation of mixed-ligand iron(II) complexes directly from phen, pdon, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

There were only traces of the mixed-ligand **5** while the second mixed-ligand complex **6** was not possible to detect under analytical HPLC conditions described in this report.

To be able to prepare the mixed-ligand complexes **5** and **6** we decided to monitor the composition of the reaction mixture and the ligand exchange process over 2 months by HPLC/PDA and LC-ESI MS. The studies were performed in two ways: (1) by monitoring the effect of the ligand ratio to the synthesis of the mixed-ligand complexes from the ligands and (2) by monitoring the exchange of the ligands in the homo *tris* $[\text{Fe}(\text{phen})_3]^{2+}$ complex, **3** by pdon.

3.1.1. The effect of phen:pdon ratio to formation of the mixed-ligand complexes. The ratio between phen and pdon was varied ($n(\text{phen}) : n(\text{pdon}) : n(\text{Fe(II)}) = 1 : (0.5-2.5) : 1$ and $n(\text{phen}) : n(\text{pdon}) : n(\text{Fe(II)}) = 2 : (0.5-2.5) : 1$). The amount of the iron(II) sulfate was kept constant. Four iron(II) complexes, **3**, **4**, **5**, and **6**, formed immediately upon mixing of the ligands and iron(II) sulfate (scheme 1). The ratio between the complexes in the mixture changed, at first the mixed-ligand complexes formed, **5** and **6**, and their amount increased (figure 1). Later the amount of **3** increased in solution and **4** precipitated. Finally, the mixed-ligand complexes **5** and **6** disappeared upon complete precipitation of homo *tris* $[\text{Fe}(\text{pdon})_3]^{2+}$, **4** (regardless the counter ion used, e.g. SO_4^{2-} or PF_6^-) and the mother liquor contained homo *tris* $[\text{Fe}(\text{phen})_3]^{2+}$, **3** and traces of **4**.

3.1.2. The effect of ligand exchange to formation of the mixed-ligand complexes. Pdon was added to aqueous solution of **3** in ratios $n([\text{Fe(II)}(\text{phen})_3]^{2+}) : n(\text{pdon}) = 1 : (0.5-2.5)$. The composition of the mixtures was monitored; pdon replaced phen forming mixed-ligand **5** and **6** regardless of the ratio used.

Therefore, the only possible way to isolate the products was chromatography. The complexes were separated by taking advantage of HPLC/PDA and HPLC/ESI-MS and by use of the Develosil RP-AQUEOUS (C30) column.

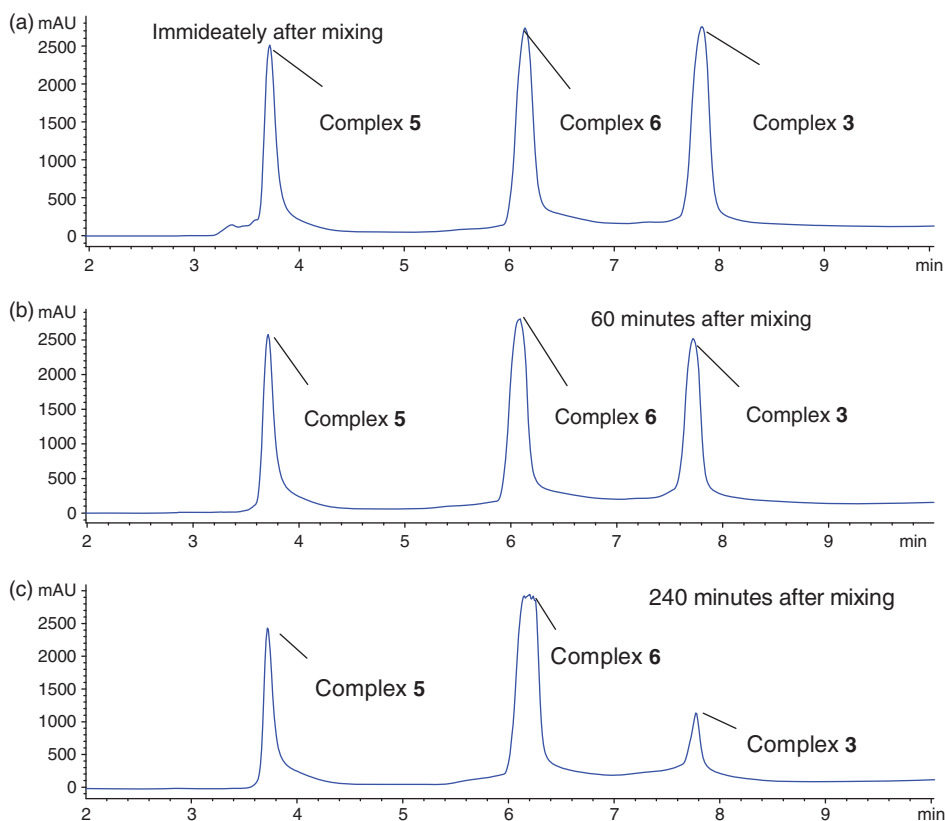


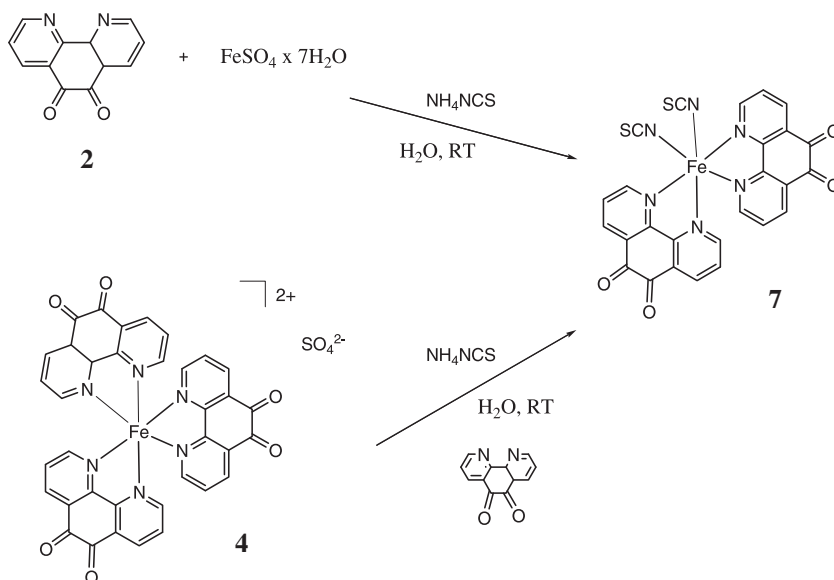
Figure 1. The chromatogram of the formed products upon mixing of the components $n(\text{phen}) : n(\text{pdon}) : n(\text{Fe(II)}) = 2 : 2 : 1$; (a) immediately after mixing, (b) after 60, and (c) after 240 min.

3.2. Synthesis of 7

The formation of **7** was straightforward (scheme 2). It was prepared and isolated according to the methods described for preparation of **4** [16]. Even though **7** could be prepared either directly from the ligands or by ligand replacement from **8**, we preferred to prepare from the ligands. The product was isolated by filtration of the precipitated solid. The purity of isolated **7** was confirmed by HRMS. The presence of $[\text{Fe}(\text{pdon})_3]^{2+}$ as the impurity was possible to determine by MS. We studied the methanol, ethanol, and acetonitrile solutions of **7** by ESI-MS and observed traces of **8** ions and its fragments only in acetonitrile solutions. In methanol or ethanol only the peaks corresponding to **7** were present and no rearrangements or impurities were observed.

3.3. Stability of the analytical solutions

The stability of the ligand solutions (pdon and phen) and homo *tris* complexes ($[\text{Fe}(\text{phen})_3]^{2+}$, **3** and $[\text{Fe}(\text{pdon})_3]^{2+}$, **4**) were monitored. The solutions were stable within 24 h, more precisely, if the solutions were captured in a capped volumetric flask on a laboratory bench under normal lighting and temperature they could be stable



Scheme 2. Formation of 7 directly from pdon (2), iron(II) sulfate, and ammonium thiocyanate or from 4 and ammonium thiocyanate.

for longer. Solutions of the mixed-ligand complexes were not stable and the ratio between the components in the mixtures changed within hours.

3.4. Specificity/selectivity

The PDA 3-D chromatogram (figure 2) demonstrates a good separation of the complexes. A wavelength of 254 nm was the most effective compromise to accomplish detection and quantification of the components in the mixture. The complexes were resolved from each other, resolution values for 5 and 6 were above 2. Precision, < 1% (repeatability and reproducibility), was performed from 5–10 replicates confirming that the method has acceptable precision.

3.5. Mobile-phase methanol–water versus acetonitrile–aqueous KPF6

Ion pair reverse chromatography is the chromatography of choice for separation of the metal complexes when MS detection is not required. Formation of solvent cluster ions occurs frequently due to association of solvent with cations and anions, resulting in the complex ESI mass spectra. The cluster ions could be eliminated by changing the MS conditions (de-clustering effect of an additional flow of a sheath gas or increasing the sampler skimmer cone voltage of the ESI interface) but those changes decrease the sensitivity of the detection. The better way is to completely eliminate the formation of clusters by varying the composition of the mobile phase.

The reverse phase separation method using methanol:water solvent system was chosen since there was no need for additional purification. IP RP separation using acetonitrile and aqueous 0.001 mol L^{-1} KPF₆ solvent system is the source for the

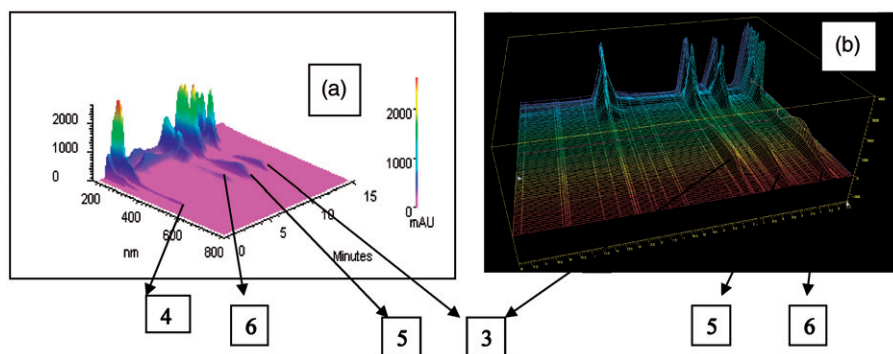


Figure 2. Typical 3-D HPLC-UV chromatogram of the mixture of iron(II) complexes **3**, **4**, **5** and **6** eluted by: (a) $\text{KPF}_6/\text{H}_2\text{O}$ and ACN as mobile phases. Sample: 10 mg of crude mixture of iron(II) complexes dissolved in 1 mL of ACN; injection: 30 μL ; mobile phases: two components gradient: (A) 0.001 mol L^{-1} KPF_6 in H_2O ; and (B) ACN; flow: 1 mL min^{-1} ; detection: UV at 254, 267, and 509 nm; temperature: 23°C; column: Develosil RP-Aqueous [C30] 5 μm , 150 \times 4.6 mm; run time: 20 min, gradient: from 80% solvent A (0.01 mol L^{-1} KPF_6) at 0 min to 50% in 12 min changing back to 80% upon 3 min, and (b) methanol and H_2O as mobile phases. Sample: 10 mg of crude mixture of iron(II) complexes dissolved in 1 mL of milli-Q water; injection: 30 μL ; mobile phases: two components gradient: (C) milli-Q H_2O ; and (D) methanol; flow: 0.5 mL min^{-1} ; detection: UV at 254, 267, and 509 nm; temperature: room; column: Develosil RP-Aqueous [C30] 5 μm , 150 \times 4.6 mm; run time: 20 min, gradient: from 85% solvent C (milli-Q water) at 0 min to 60% in 7 min changing to 50% at 8th minute, from 8 to 19 min solvent C slowly lowered to 30% and finally in 1 min returned to the beginning value of 85% of C in 3 min.

difficulties in assignation by MS and also requires the additional step of salt extraction. Mixed-ligand complexes that contain pdon form clusters with polar protic solvents, thus causing more complex assignment of the spectra, but the solvent-complex ion clusters actually were helpful in confirmation of the proposed formulae of the mixed-ligand complex ions (figures 3 and 4 and table 1).

3.6. Separation of **5** and **6**

Mixed-ligand iron(II) complexes with ligands that are only slightly different are impossible to prepare pure and require purification. Unfortunately, they cannot be purified by standard chromatography [17] since they tend to adsorb irreversibly on silica and alumina. Mudasir *et al.* [18] published the method for the mixed-ligand complex preparation by (4,7-diphenyl-1,10-phenanthroline) ligand substitution from $[\text{Fe}(\text{phen})_3]^{2+}$. The crude products were purified by preparative HPLC (Develosil ODS column 10 μm , 250 \times 30, 5 mm) to give pure mixed-ligand complexes. Upon separation of the mixture by HPLC, immediate evaporation of acetonitrile from the solution was needed to avoid possible substitution for the solvent or dissociation of the complexes. Mudasir performed extractions of the separated complexes to remove excess salts from the mobile phases.

Following those results, we developed the method for separation and characterization of the mixed-ligand phen and pdon iron(II) complexes by using complementary chromatographic methods such as HPLC/MS and HPLC/DAD for analytical and semi-preparative separations. The PDA detector has capability to acquire and store a great amount of spectral data from the UV absorbing compounds, thereby making

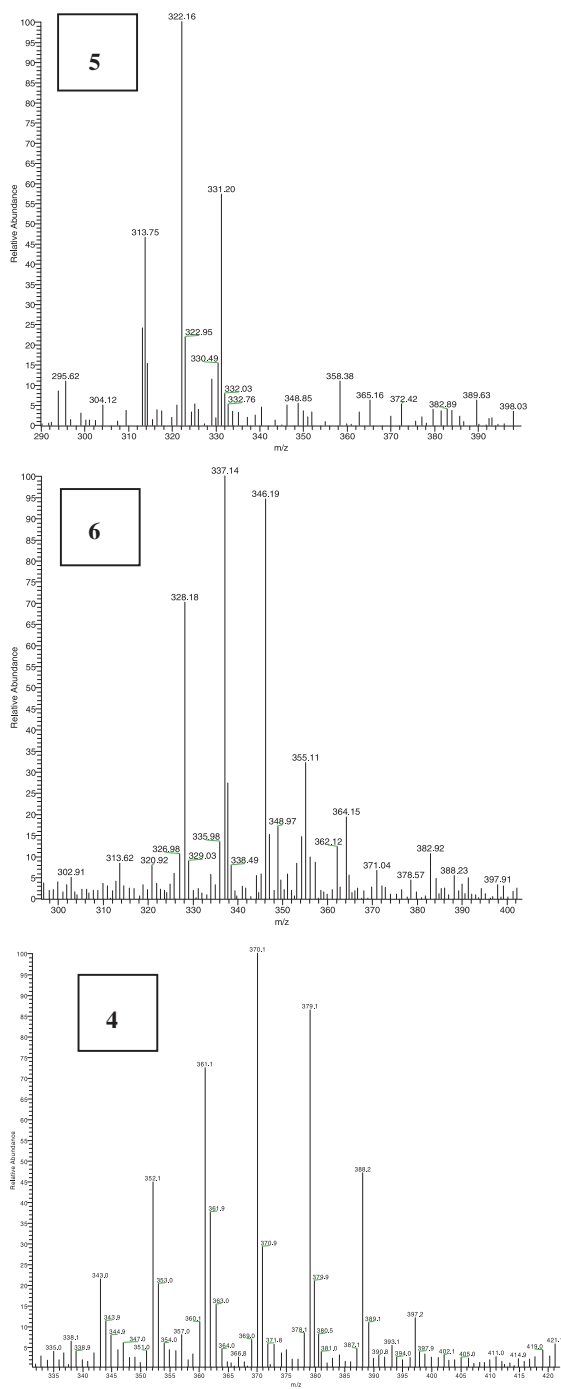


Figure 3. The water clusters of the separated complex ions of 4, 5, and 6.

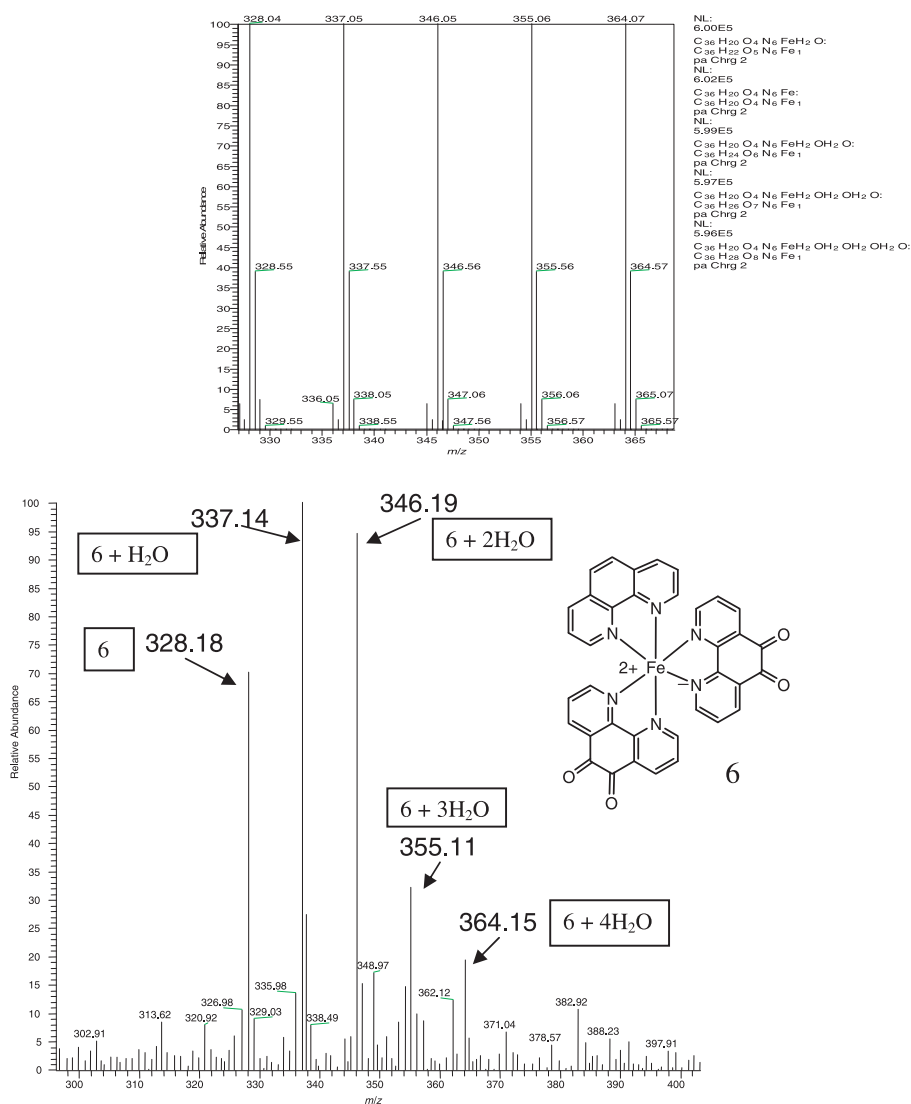
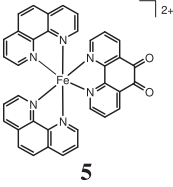
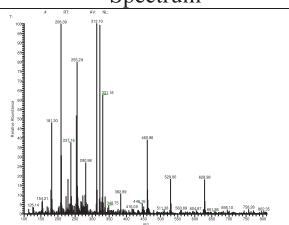
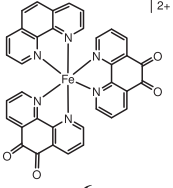
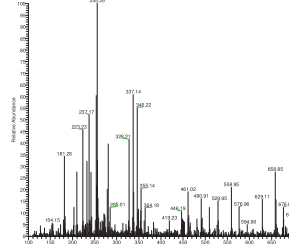


Figure 4. The theoretical (top) [19] and the experimental (bottom) patterns of the peak at m/z 328.18 corresponding to $[\text{Fe}(\text{phen})(\text{pdon})_2]^{2+}$ of **6** and its water clusters at m/z 337.14 assigned as $[\mathbf{6}+(\text{H}_2\text{O})]^{2+}$; 346.19 assigned as $[\mathbf{6}+(\text{H}_2\text{O})_2]^{2+}$; 355.11 assigned as $[\mathbf{6}+(\text{H}_2\text{O})_3]^{2+}$; and 364.15 assigned as $[\mathbf{6}+(\text{H}_2\text{O})_4]^{2+}$.

possible both spectral identification and individual analysis of the peak homogeneity/purity of each chromatographic peak.

The Develosil RP-Aqueous [C30] $5\ \mu\text{m}$, $150 \times 4.6\ \text{mm}$ column was the column of choice since its stationary phase is very stable even under 100% aqueous conditions as well as from 2 to 8 pH. Conventional stationary phases such as C18 under aqueous conditions collapse and fold on the silica gel surface to escape from water but the use of C30 diminished the collapsing effect. The collapsing of the ligands leads to changes in retention times and poor reproducibility, especially when iron complexes were separated, the presence of “the naked silica-gel surface” led to irreversible adsorption.

Table 1. The major peaks (m/z) and ESI-MS spectra of $[\text{Fe}(\text{phen})_2(\text{pdon})]^{2+}$ and $[\text{Fe}(\text{pdon})_2(\text{phen})]^{2+}$.

Complex ion	Mr	Major peaks m/z	Spectrum
 5	626	313.11 $[\text{Fe}(\text{phen})_2\text{pdon}]^{2+}$ 322.17 $[\text{Fe}(\text{phen})_2(\text{pdon})(\text{H}_2\text{O})]^{2+}$ 331.19 $[\text{Fe}(\text{phen})_2(\text{pdon})(\text{H}_2\text{O})_2]^{2+}$	
 6	656	328.18 $[\text{Fe}(\text{phen})(\text{pdon})_2]^{2+}$ 337.14 $[\text{Fe}(\text{phen})(\text{pdon})_2(\text{H}_2\text{O})]^{2+}$ 346.19 $[\text{Fe}(\text{phen})(\text{pdon})_2(\text{H}_2\text{O})_2]^{2+}$ 355.11 $[\text{Fe}(\text{phen})(\text{pdon})_2(\text{H}_2\text{O})_3]^{2+}$ 364.15 $[\text{Fe}(\text{phen})(\text{pdon})_2(\text{H}_2\text{O})_4]^{2+}$	

Efficient chromatography and high sensitivity were achieved by using acetonitrile and aqueous 0.001 mol L^{-1} KPF_6 as the mobile phase. The separations were monitored at 254, 267, and 509 nm. The free ligands adsorb at the first two wavelengths and the maximum of the MLCT band varies between 450 and 550 nm depending on the ligands in the mixed-ligand complexes. The 3-D chromatogram of **3–6** eluted on analytical HPLC under the conditions described in the experimental section is shown in figure 2. From the chromatogram the retention times R_t of the complexes increase as the hydrophobicity of the complex also increases, namely the number of phen groups rises from 0 to 3.

The products were successfully separated by using gradient elution. The fractions A1 (retention time 7:4 to 8:1 min) and A2 (retention time 9:0 to 10:2 min) were collected with **6** and **5**. The immediate evaporation of the solvent from the solution (quick lyophilization) was required to prevent ligand exchange.

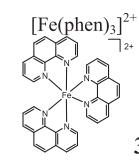
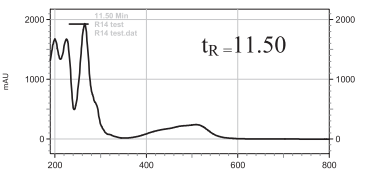
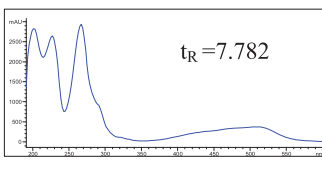
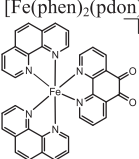
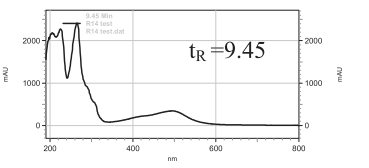
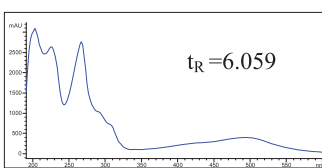
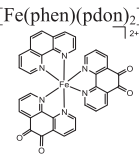
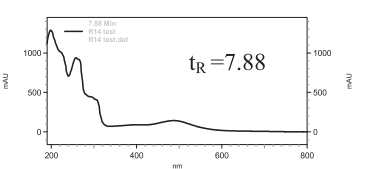
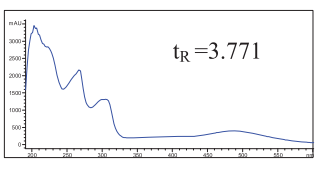
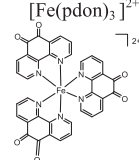
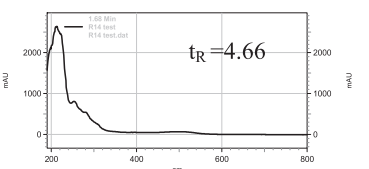
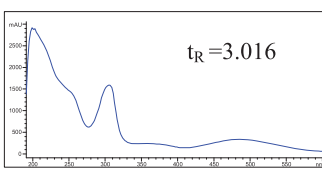
Since the complexes were separated but the KPF_6 retained, it was necessary to extract the product to remove the salt. Therefore, we developed a method for separation of the mixed-ligand complexes that did not involve salt but only water and methanol. The separation was accomplished by using previously mentioned Develosil RP-Aqueous [C30] column (figure 2b).

The complexes were separated and the UV-Vis spectra for the complexes are shown in table 2.

3.7. Identification of the components by LC-MS

ESI-MS, the softest desorption/ionization method, is a powerful tool for characterization of metal complexes as well as to obtain information about the molecular weight, the charge, and the isotope distribution of molecules. It is ideally suited to investigate the coordination behavior of transition metal complexes [20, 21]. Posey *et al.* [22]

Table 2. UV-Vis spectra of **3**, **4**, **5**, and **6** upon separation.

Complex ion	acetonitrile/0.001M aq. KPF ₆	methanol/water
 <p>3</p>	 <p>$t_R = 11.50$</p>	 <p>$t_R = 7.782$</p>
 <p>5</p>	 <p>$t_R = 9.45$</p>	 <p>$t_R = 6.059$</p>
 <p>6</p>	 <p>$t_R = 7.88$</p>	 <p>$t_R = 3.771$</p>
 <p>4</p>	 <p>$t_R = 4.66$</p>	 <p>$t_R = 3.016$</p>

showed that ESI produces gas-phase clusters containing transition metal ion complexes analogous to those found in solution. This technique provided us with a view of the complex composition (combined with the NMR results) and the complex behavior in the various solvents. Both ligands, phen and pdon, the reaction mixtures, and the complexes, **3**, **4**, **5**, **6**, and **7**, were studied by ESI-MS, HRMS, and LC-MS. The ligands and the complexes were studied in polar protic (water, methanol, ethanol) and aprotic (acetonitrile) solvents.

ESI-MS studies of the 1,10-phenanthroline-5,6-dione and **3**, **4**, and **7** showed that the polar protic solvents formed clusters with free pdon or pdon bound in the complex ions [23]. The foundation for this kind of solvent cluster was most likely hydrogen-bond formation between the oxygen atoms (keto groups of the pdon) and the polar solvent (water, methanol, ethanol). An example is the observation of the solvent (methanol) – ligand (pdon) clusters when the complexes were dissolved in methanol. The number of solvent molecules in the cluster corresponded to the number of keto groups present in the complex (table 2). When mixtures of polar solvents were used mixed clusters with solvents were observed. Iron(II) complexes with 1,10-phenanthroline such as $\text{Fe}(\text{phen})_2(\text{NCS})_2$ (**8**) and $[\text{Fe}(\text{phen})_3]^{2+}$ did not show any association with solvent molecules.

The mixed-ligand complexes were prepared in water and when the separation of the complexes was performed in water/methanol then water clusters of each complex were

Table 3. The UV-Vis maxima in nm of **3**, **4**, **7**, **8** and ligands **1** and **2** in MeOH and ACN.

Compound	UV-Vis maxima (nm)	
	MeOH	ACN
phen (1)	229, 263, 309, 323	229, 263, 309, 323
Fe(phen) ₃ (PF ₆) ₂ (3)	225, 266, 477, 509	225, 266, 477, 509
Fe(phen) ₂ (NCS) ₂ (8)	225, 266, 473, 509	225, 266, 473, 509
pdon (2)	233, 245, 295	251, 255, 292, 305, 369
Fe(pdon) ₃ (PF ₆) ₂ (4)	235, 252, 298, 305, 483	299, 311, 368, 471
Fe(pdon) ₂ (NCS) ₂ (7)	229, 250, 295, 480	247, 296, 361, 471

observed (figure 3). The doubly charged ions of **5** and **6** were observed. Comparison of the theoretical and experimental patterns of the peaks in the clusters confirmed the proposed structures (figure 4). When the separation was achieved using acetonitrile/aq. KPF₆ as mobile phases then salt clusters were observed and assignment of the MS spectra was difficult.

3.8. Absorption spectra

The iron(II) complexes have been shown to be essentially diamagnetic. Williams suggested that the intense absorptions in the visible region for diamagnetic iron(II) complexes arise from excitation of a 3d electron to a level which can participate or couple with the empty levels of the ligand [24, 25]. Absorption in the region between 250 and 350 nm belongs to intra-ligand $\pi \rightarrow \pi^*$ absorption or $n \rightarrow \pi^*$. However, absorption maxima of the metal chelates are shifted toward longer wavelength compared with that of free ligand, proving the coordination of ligand to iron(II). The coordination is also confirmed by the presence of absorption of iron(II) in the visible region between 450 and 550 nm due to MLCT, the transitions of core $\pi^2 t_2^6 \rightarrow \pi^2 t_2^5 \pi^*$. It is a group of bands ascribed to Laporte-allowed transitions from t_2 orbitals of metal ion to orbitals of the heterocyclic ligands ($t_2 \rightarrow \pi^*$). This MLCT absorption is broad and shows vibrational structure. Because the phen system is characterized by two low-lying unoccupied molecular orbitals of comparable energies [26], the visible MLCT absorption of complexes should contain two kinds of very closely located MLCT transitions, $d \rightarrow \pi(8)$ and $d \rightarrow \pi(9)$ and the order of these in the complex relative to the free ligand is a matter of some interest [27].

UV-Vis spectra of aromatic ketones are composed of $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ absorptions which show opposite solvent effects; going from a nonpolar to a polar solvent, the $\pi \rightarrow \pi^*$ absorption should undergo a bathochromic shift, while the $n \rightarrow \pi^*$ absorption undergoes a hypsochromic shift [28]. This contradictory behavior of absorptions with changes in solvent polarity is of diagnostic importance to distinguish between the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions in carbonyl compounds and also to help in assignment of absorptions of mixed-ligand complexes.

Absorption spectra of our complexes and free ligands were taken in acetonitrile and methanol. From the results (table 3) we conclude that free phen and complexes with phen as the only organic ligand are not sensitive to changes of solvent polarity. Both $\pi \rightarrow \pi^*$ transitions and MLCT in Fe(II) complexes with phen (**3** and **4**) are not

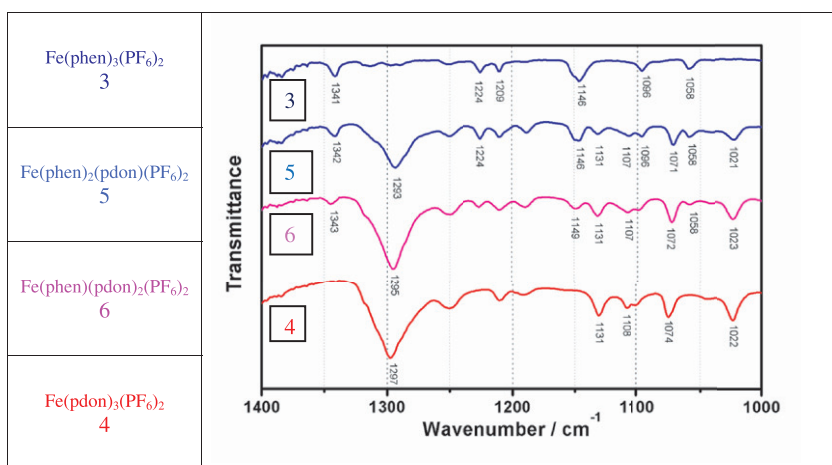


Figure 5. IR bands of **3**, **4**, **5**, and **6** in KBr pellets in the region $1400\text{--}1000\text{ cm}^{-1}$.

influenced by solvent polarity; absorption bands at 225, 266, or 509 remained unchanged.

The results presented in table 3 indicate that the interaction of the ligand with solvent is transferred to the interaction of the whole complex if only one kind of ligand is present. When the ligand with carbonyl is present in the mixed-ligand complex then changes of polarity of the solvent could be used in assignment of the mixed-ligand complex composition (table 1).

3.9. Vibrational spectra

Infrared spectroscopy is a satisfactory method for identification of mixed-ligand iron(II) complexes since the IR spectra of free ligands and complexes could be easily distinguished. The spectra of the homo *tris* $[\text{Fe}(\text{pdon})_3]^{2+}$ complex and mixed-ligand iron(II) complexes **5** and **6** were far simpler than the free ligands, especially in the $900\text{--}600\text{ cm}^{-1}$ region, and clear difference in the band positions and intensities were observed in the $1400\text{--}1000\text{ cm}^{-1}$ region. Representative spectra of **3**, **4**, **5**, and **6** in that region are shown in figure 5. Bands at $\sim 836\text{ cm}^{-1}$ and 557 cm^{-1} belong to PF_6^- and were present in all spectra, confirming that PF_6^- was not involved in coordination but served simply as a counter ion. The NCS^- ion was involved in the iron(II) coordination and its band positions changed upon complexation. Thus in **8**, $(\text{Fe}(\text{phen})_2(\text{NCS})_2)$ bands were at 2073 and 2060 cm^{-1} while in **7**, $(\text{Fe}(\text{pdon})_2(\text{NCS})_2)$ bands were at 2086 and 2054 cm^{-1} . In NH_4NCS the band was at 2067 cm^{-1} .

3.10. $^1\text{H-NMR}$

Assignment of various peaks to proton is either straightforward or analogous to similar iron complexes [29] or analogous to ruthenium complexes that have been extensively studied [30]. Proton NMR shifts were in agreement with those obtained for

Table 4. The ^{13}C -NMR shifts of the 1,10-phenanthroline-5,6-dione carbons of **4**, **5**, **6**, and **7** in DMSO.

Carbon	Fe(pdon) ₂ (NCS) ₂ (7)	Fe(pdon) ₃ (PF ₆) ₂ (4)	Fe(phen)(pdon) ₂ (PF ₆) ₂ (6)	Fe(phen) ₂ (pdon)(PF ₆) ₂ (5)	Pdon (2)
C=O	174.48	174.5	174.71	174.59	178.21
C _{6'}	158.26	158.3	158.69	158.79	152.75
C _{5'}	129.96	131.98	131.78	131.83	129.55
C _{2'}	158.19	158.2	158.36	158.40	154.79
C _{3'}	136.50	136.72	136.27	136.45	136.12
C _{4'}	128.32	128.9	128.69	128.79	125.69

mixed-ligand iron(II) complexes of phen and dip (4,7-diphenyl-1,10-phenanthroline) [13] for the phen part of the complex.

Our ^1H -NMR data indicated clearly that the complexes are diamagnetic. Downfield shift (δ) in phen protons occurred upon chelation of the ligands to iron(II) except for the 2, 9 protons. This deshielding effect could be attributed to decrease in electron density at individual carbon nuclei as a result of interaction with the positive iron(II). The effect doubled for H_{4,7} in phen (shift of 0.50 ± 0.06 ppm) in regard to pdon (shift of 0.25 ± 0.02 ppm). On the other hand, H_{2,9} showed a dramatic upfield shift in the range from 1.2 to 1.3 ppm in phen and pdon due to shielding cone of the heterocyclic ring of the adjacent ligand. Castellano *et al.* [29] explained this effect as a diamagnetic anisotropic effect of the aromatic ring of the adjacent ligand. The ^{13}C -NMR data for pdon of **5**, **6**, and **7** in DMSO are presented in table 4 together with those for the corresponding homo *tris* [Fe(pdon)₃]⁺ complex and the free ligand for comparison.

4. Conclusion

Our results demonstrate that we have developed two reverse phase HPLC methods capable of separating mixed-ligand complexes (**5** and **6**) of iron(II) containing 1,10-phenanthroline and 1,10-phenanthroline-5,6-dione as ligands. IC RP HPLC could be efficiently replaced by the RP HPLC method for separation of the mixed-ligand iron(II) complexes when appropriate column (Develosil RP-Aqueous [C30]) and the chromatographic conditions were applied. In this way, we completely eliminated formation of salt clusters by varying the composition of the mobile phase and the previously needed extraction upon chromatographic separation. The ESI tandem MS technique was used to determine the formulae of the complexes and to confirm the structures determined by NMR, UV-Vis, and IR.

Acknowledgments

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