SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF IRON CYCLOHEXANEDICARBOXYLIC ACID AND EXAMINATION OF pH EFFECT ON EXTRACTION IN WATER AND ORGANIC PHASES

Nilgün BECENEN1*, Gühergil ULUÇAM2, Özlen ALTUN2

1 Trakya University, Vocational College of Technical Sciences, 22030 Edirne
2 Trakya University, Department of Chemistry, 22030 Edirne
*Corresponding author: e-mail: nilgunbecenen@trakya.edu.tr

Received (Alınış): 06 January 2017, Accepted (Kabul): 06 February 2017, Online First (Erken Görünüm): 10 March 2017, Published (Basım): 15 June 2017

Abstract: In this study, iron cyclohexanedicarboxylic acid was synthesized under refluxing conditions. The structural characterization of the compound was performed by using physico-chemical and spectroscopic methods. The effect of pH on extraction percentage (%E) in water and organic phase was investigated with the extraction method. At the end of the experiment, most of the Fe²⁺ ions in the water phase at pH=7.5 passed into the organic phase. Above this pH, the extraction percentage decreased because of the transition from water phase to organic phase. Iron cyclohexanedicarboxylic acid was extracted with ether, petroleum ether, benzole and carbon tetrachloride from aqueous solution and microbial activities of the complex were studied. The activity data showed that iron cyclohexanedicarboxylic acid has an important antibacterial and antifungal activities.

Key words: Fe (II), cyclohexanedicarboxylic acid, synthesis, extraction.

Introduction

Low molecular weight cyclic organic acids are liquids with disturbing odor and high viscosity, whereas high molecular weight cyclic acids are either liquids with light odor and low viscosity or solids. They are soluble in many organic solvents (Battersby et al. 1968, March 1985). Many studies on cis and trans-1,2-cyclohexanedicarboxylic acid have previously been published so far (Björkling et al. 1985, Reiznaut et al. 2009, Altun et al. 2010, Chen et al. 2014). For instance, the Hunsdiecker reaction of silver salts of cis- and trans-1,2-cyclohexanedicarboxylic acid was investigated by Abell (1957). Kamino et al. (1996) reported that the molecular packing was affected by the shape of polycarboxylate molecules in the case of phthalate, cis-1,2-cyclohexane dicarboxylate and 1,1-cyclohexanedicarboxylate. Koster et al. (2001) examined hyperbranched synthetic polyesteramides synthesized by the polycondensation of trifunctional diisopropanolamine and dysfunctional anhydrides of succinic acid, glutaric acid, cis-1,2-cyclohexanedicarboxylic acid and phthalic acid. We considered that if different metal salts of these compounds are synthesized, then they may be used in many different areas. For this purpose, we started a study with the aim of creation of iron salt of cyclohexanedicarboxylic acid to examine all aspects of this compound. We also investigated the extraction of the obtained iron cyclohexanedicarboxylic acid with pH effect between water and organic phase in addition to its antibacterial and antifungal activities.

Materials and Methods

Materials and Apparatus

Cyclohexanedicarboxylic acid (Aldrich) and diethylether (Merck) were all organic reagent grade, and FeSO₄.7H₂O (Merck) was analytical reagent grade.
Elemental analyses for C and H were obtained on dried samples using a Perkin Elmer 2400 elemental analyzer. FT-IR spectra were determined on a Perkin Elmer BXII spectrometer as KBr pellets in the frequency range 400-4000cm\(^{-1}\). UV/Vis spectra were recorded at 25°C with a Shimadzu UV-1700 Pharma spectrophotometer in the wavelength range 200-800nm. XRD analyses were investigated in Shimadzu XRD-6000.

A calibrated Metrohm 654 digital pH meter with a pH glass electrode mounting was employed for pH measurements. The pH meter was adjusted before use with pH 4 and 7 Metrohm AG CH 9100 Hersau Buffers.

Synthesis of iron cyclohexanedicarboxylic acid

Method I. Two step substitution reaction

Step I: 20g (0.117mol) of cyclohexanedicarboxylic acid was dissolved in 150ml ether and placed on a magnetically-stirred heater. The system was heated and stirred to reflux while 10g (0.25mol) NaOH in 40ml water solution was added dropwise within 20-24 minutes. The reaction mixture was poured into an extraction funnel. The pH was adjusted to 7 by adding NaOH solution. Aqueous phase was removed and washed with ether, evaporated slowly and a white powder, identified as sodium dicarboxylic acid, was obtained (1) [Color: White. Yield (%): 82 %. M.P.: 232.6°C. FT-IR bands (cm\(^{-1}\)): 2878cm\(^{-1}\) (\(\nu\)CH)), 1738-1550cm\(^{-1}\) (\(\nu\)carboxyl anions), 1475cm\(^{-1}\) (\(\nu\)CO)]. UV/Vis bands (nm): 302.

Step II: 5g (0.023mol) sodium dicarboxylic acid was dissolved in 20ml water and placed in a magnetically stirred 250ml three necked flask which was equipped with a thermometer, a condenser and an additional funnel filled with 5g (0.018mol) FeSO\(_4\).7H\(_2\)O solution in 7ml water. FeSO\(_4\).7H\(_2\)O solution was added dropwise at 85-90°C within one hour. At the end of the reaction, solid brown iron dicarboxylic acid was filtered and washed with distilled water several times to remove water soluble material. The remaining solid residue was kept under vacuum producing a brown solid identified as iron dicarboxylic acid (2) [Color: Brown. Yield (%): 84. M.P.: 265°C. Elemental Analyses (%): Calc. C 42.55, H 4.23. Found: C 42.6, H 4.4. FT-IR bands (cm\(^{-1}\)): 2944cm\(^{-1}\) (\(\nu\)CH)), 1593-1532cm\(^{-1}\) (\(\nu\)carboxyl anions), 1436-1398cm\(^{-1}\) (\(\nu\)CO)]. UV/Vis bands (nm): 290, 511. XRD (2\(\theta\)) : 5.2300, 14.9200 and 19.5600.

Method II: One step substitution reaction

A solution of 20g (0.11 mol) cyclohexanedicarboxylic acid in 150ml ether was placed in a magnetically stirred three necked flask on which a thermometer, a condenser and an additional funnel were attached. 10g (0.25mol) NaOH in 40ml water was placed in the additional funnel and dropwise addition was begun after internal temperature reached to 40-45°C with external heating. After NaOH addition was completed, 5g (0.018mol) FeSO\(_4\).7H\(_2\)O solution in 7ml water was placed in the additional funnel and the previous operation was repeated at 85-90°C. Mixture was stirred for about one hour. During the progress of the reaction ether was lost gradually from the system. Finally, the liquid phase was filtered giving brown residue which was washed with distilled water to get rid of the water soluble impurities. The brown solid was oven dried giving a compound with a spectrum consistent with the spectrum of the compound obtained in Method I.

We adjusted the pH value of sodium dicarboxylic acid solution to 7 before treating it with FeSO\(_4\).7H\(_2\)O solution in both methods to obtain the maximum yield. At pH values higher than 7 complications occur in the reaction as a result of a reaction between hydroxyl and iron ions (3) [Color: Brown. Yield (%): 89 %. M.P.: 265.2°C. Elemental Analyses (%): Calc. C 42.53, H 4.26. Found: C 42.6, H 4.4. FT-IR bands (cm\(^{-1}\)): 2944cm\(^{-1}\) (\(\nu\)CH)), 1605-1558cm\(^{-1}\) (\(\nu\)carboxyl anions), 1400cm\(^{-1}\) (\(\nu\)CO)]. UV/Vis bands (nm): 290, 511. XRD (2\(\theta\)) : 5.2300, 14.9200 and 19.5600.

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The extraction of iron cyclohexanedicarboxylic acid in water and organic phases with pH effect

The aqueous solution of 5ml of 0.1N NaOH for pH=4 to 9.5 and solution of 1.3g (0.078mol) cyclohexanedicarboxylic acid in 10ml flask were stirred in ten separate flasks. Afterwards, 10ml of aqueous solution including 1mg/ml Fe$^{2+}$ was added to this mixture, and after stirring for one hour at ambient temperature, the mixture was left for 24 hours. Composed solutions were separately placed in an extraction flask, and the amount of Fe$^{2+}$ ions in organic and water phases was determined with atomic absorption with Unicam model 929, Aaglame (Yang et al. 1991, İnci 2002).

The percentage of the extraction was calculated with the following formula:

\[
%\ E = (\alpha+ V_w / V_o) \cdot 100
\]

\[
\alpha = C_o / C_w
\]

Where \(\%\ E\) = Extraction percentage, \(V_{org}\) = Volume of the organic phase (ml), \(V_w\) = volume of water phase, \(\alpha\) = sharing constant, \(C_{org}\) = Fe$^{2+}$ concentration in organic phase (mg/ml), \(C_w\) = Fe$^{2+}$ concentration in water phase (mg/ml).

In all samples, 5ml 0.1N NaOH solution, 10ml FeSO$_4$.7 H$_2$O (1mg/ml Fe$^{2+}$) solution and 10ml ether solution in proportion 1.3g (3.7x10$^{-3}$mol) iron dicarboxylic acid were used.

Biological activity procedure

The antibacterial and antifungal activities of the obtained complexes is tested by using agar well-diffusion susceptibility test (Shaukat et al. 1980) is carried out against Gram-positive bacteria (Bacillus subtilis Ehrenberg, Staphylococcus aureus Rosenbach) and Gram-negative bacteria (Escherichia coli T. Escherich, Pseudomonas aeruginosa (Schroeter) Migula) and the agar tube dilution protocol method (Kazmi et al. 1991) is applied against Candida albicans (Berkhout), Candida glabrata (Anderson) Mey & Yarrow and Fusarium solani (Mart.) Sacc), respectively.

Results and Discussion

Synthesis and analyses

In the present study, the synthesis and the properties of a coordination compound of cyclohexanedicarboxylic acid and Fe (II) were studied. Two methods were employed for the synthesis and high yields were found to be fairly close with both methods. Spectroscopic methods showed the possible structure of iron dicarboxylic acid. Iron dicarboxylic acid is a brown solid. The solubility in different solvents and the results of the elemental analysis are given in Table 1 and Table 2. Elemental analysis determination is in good agreement with the general formula for the complex. The elemental analysis demonstrated that the obtained iron cyclohexanedicarboxylic acid has 1:1 stoichiometry. Complex is solid, insoluble in water but soluble in some organic solvents such as benzene, ether, Acetone, CCl$_4$ and CH$_3$COOH.

The FT-IR spectra of the complex (Figure 1) demonstrates two bands centered at 1593 and 1532 cm$^{-1}$ are attributed to the (\(\nu_{\text{carboxyl anions}}\)) vibrations. The band at about 2944 cm$^{-1}$ is assigned to the absorption of the (\(\nu_{\text{CH}}\)) vibrations and the (\(\nu_{\text{CO}}\)) stretching of the complex are found in the frequency range about 1593 and 1532 cm$^{-1}$. FT-IR bands of cyclohexanedicarboxylic acid (2878 cm$^{-1}$ (\(\nu_{\text{CH}}\)), 1738-1550 cm$^{-1}$ (\(\nu_{\text{carboxyl anions}}\)), 1475 cm$^{-1}$ (\(\nu_{\text{CO}}\)) are shifted to lower frequencies in the complex.

![Figure 1. The FT-IR Spectrum of iron cyclohexanedicarboxylic acid](image-url)
The UV/Visible spectrum of cyclohexanedicarboxylic acid molecule shows an absorption band at 302 nm, whereas the complex demonstrates absorption bands at 290 and 511 nm. The band at 290 nm is attributed to n-π* and π-π* transitions, while the band at 511 nm is caused by the electronic transitions both n-π* and π-π* and also charge transfer transition arising from π electron interactions between the metal and ligand that involves either a metal-to-ligand or ligand-to-metal electron transfer.

The XRD-Powder Pattern spectrum (Figure 2) of the complex is recorded by an X-Ray diffractometer and the unit cell parameters are calculated from 2θ values with the help of a computer. Powder XRD pattern of complex consist of three important peaks in the range 5-50°C (2θ). The inter planar spacing (d) is calculated from the positions of intense peaks using Bragg’s relationship. The 2θ values with maximum intensity of the peaks for Fe (II) complex were found to be 5.2300, 14.9200 and 19.5600 (2θ) which corresponds to d:16.97784, 5.93296 and 4.53476, respectively.

Thermogravimetric analysis determined the localization of the central cation in the complex molecule. Thermogravimetric analysis of iron cyclohexanedicarboxylic acid indicated two steps for decomposition and the amount of water in the composition of the molecule. Experimental thermolysis is given in Table 1 and Figure 3.

The decomposition of two carboxyl groups can be monitored from the thermogram. Up to 181°C, a water molecule leaves the compound, then between 306-724°C, the organic component decomposes. The remaining compound over 724°C was determined as FeO. After these observations we concluded that the structure of iron cyclohexanedicarboxylic acid is [Fe(C₈H₁₀O₄)]. 7H₂O. The structural formula of the compound is shown below.

![Structural formula of the compound](Scheme 3)

**Extraction results**

Table 2 shows the extraction results. The graphics which show transition related to pH of the sharing constant (α) and extraction percentages (% E) of iron dicarboxylic acid for these values are given in Figure 4 and Figure 5. At the end of the experiment, most Fe²⁺ ions in the water phase at pH=7.5 passed into the organic phase, and the extraction percentage at this pH became E = 96.5%. Extraction percentages decreased at pH values above 7.5 because of the transition from water phase to organic phase, (Figure 4).

Extraction experiments were repeated with benzine, benzole and carbon tetrachloride, apart from ether, and extraction yields were found to be close to 100 % between pH = 7.5-8 (Table 2). In this way, it was proven that in addition to ether, petroleum ether, benzole and CCl₄ solvents are also suitable extraction substances for iron cyclohexanedicarboxylic acid.

![Figure 2. The XRD Spectrum of iron cyclohexanedicarboxylic acid](Image)

**Table 1. Thermogravimetric analysis data of iron dicarboxylic acid**

<table>
<thead>
<tr>
<th>Compound</th>
<th>First step decomposition</th>
<th>Second step decomposition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Te-Tf (°C)</td>
<td>Weight loss (%)</td>
</tr>
<tr>
<td>Iron Dicarboxylic acid</td>
<td>0-181</td>
<td>4.0</td>
</tr>
</tbody>
</table>
Iron Cyclohexanedicarboxylic Acid: Synthesis and Examination of Some Properties

Figure 3. TG-DTA of iron cyclohexanedicarboxylic acid

Figure 4. Transition of sharing constants of iron cyclohexanedicarboxylic acid with pH

Figure 5. Transition of extraction percentages of iron cyclohexanedicarboxylic acid with pH

Table 2. Extraction of Fe\textsuperscript{3+} in different solvents from the aqueous solution

<table>
<thead>
<tr>
<th>Sample</th>
<th>Organic Solvents (10ml)</th>
<th>pH</th>
<th>Water phase Cw (mg/ml) at the end of the extraction (1mg/ml Fe\textsuperscript{3+})</th>
<th>Organic phase Co (mg/ml) at the end of the extraction (1mg/ml Fe\textsuperscript{3+})</th>
<th>(\alpha)</th>
<th>E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petrol. ether</td>
<td>7.9</td>
<td>0.003</td>
<td>0.095</td>
<td>31.60</td>
<td>95.40</td>
</tr>
<tr>
<td>2</td>
<td>Benzoile</td>
<td>7.6</td>
<td>0.005</td>
<td>0.156</td>
<td>38.8</td>
<td>96.2</td>
</tr>
<tr>
<td>3</td>
<td>CCl\textsubscript{4}</td>
<td>7.7</td>
<td>0.002</td>
<td>0.173</td>
<td>86.5</td>
<td>98.3</td>
</tr>
</tbody>
</table>

### Biological activity results

The results for the agar well-diffusion susceptibility tests showed that the iron complex potent antibacterial activity against Gram-positive bacteria (Bacillus subtilis Ehrenberg, Staphylococcus aureus Rosenbach) and Gram-negative (Escherichia coli T. Escherich, Pseudomonas aeruginosa (Schroeter) Migula) (Table 3).

Table 3. The antibacterial activities of the iron complex and standard

<table>
<thead>
<tr>
<th>Bacteria\textsuperscript{a}</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron Complex</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>25</td>
</tr>
<tr>
<td>E. coli</td>
<td>20</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>25</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Concentration of sample is 3mg/ml DMSO, \textsuperscript{b}Inipenum, 10µg/disc

The results of the agar tube dilution protocol method showed that the iron complex has significant antifungal activity against Candida albicans (Berkhout), Candida glabrata (Anderson) Mey & Yarrow and Fusarium solani (Mart.) Sacc. (Table 4).

Table 4. The antifungal activities of the iron complex and standard.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron Complex</td>
</tr>
<tr>
<td>C. albicans</td>
<td>75</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>40</td>
</tr>
<tr>
<td>F. solani</td>
<td>70</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Incubation temperature: 27°C, \textsuperscript{b}Concentration of sample 200µg/mL of DMSO.

### Conclusion

In this study, two synthesis methods were performed and same physico-chemical properties of the product were obtained with both methods. According to spectrophotometric data, the reactions of cyclohexanedicarboxylic acid in the presence iron (II) are complexation reactions. One molecule of cyclohexanedicarboxylic acid reacts with one molecule of iron (II) and proposed structure of iron cyclohexanedicarboxylic acid is [Fe(C\textsubscript{6}H\textsubscript{10}O\textsubscript{4})]. 7H\textsubscript{2}O. Extraction experiment results showed that most Fe (II) ions in the water phase passed into the organic phase at pH=7.5 and above this pH value extraction percentages decreased because of the transition from water phase to organic phase. Extraction experiments were repeated with petroleum ether, benzole and carbon tetrachloride, and extraction yields are obtained close to 100 % between pH = 7.5-8. According to biological test results, iron cyclohexanedicarboxylic acid was found that have antibacterial affects against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa (Table 3) and antifungal affects against Candida albicans, Candida glaberata and Fusarium solani (Table 4).

### Acknowledgement

We would like to thank Prof. Dr. Ömer Zaim from Trakya University, Chemistry Department, Edirne, Turkey and dietician Figen Esen from Osman Gazi University, Faculty of Medicine, Eskişehir, Turkey for their help.

### References


