

**Process innovation
in the production of chelates
for agricultural uses**

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**PROCESS INNOVATION
IN THE PRODUCTION OF CHELATES
FOR AGRICULTURAL USES**

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Chapter one

Introduction

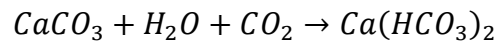
1.1 The iron and the iron chlorosis

1.1.1 The importance of iron in the plant life

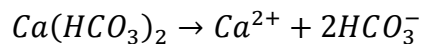
The iron is one of the most important microelements necessary to the life and development of plant because it intervenes in a chlorophyll formation and participates as catalyst in some fundamental metabolic reactions. The bioavailability of iron is related to its oxidation state, in fact the plant adsorbs the Fe^{3+} present in the soil by the roots where a reduction from Fe^{3+} to Fe^{2+} occur, thanks to the enzymatic reaction. The availability of iron in the soil is affected by many factors but it is strictly related to the pH of soil, in fact the iron presence increases with the reduction of pH [1].

Nevertheless pH between 6.8 and 7.3 guarantees a sufficient iron contribution but for values just more than 7.8 (typical of calcareous soil) the iron availability decreases with consequent problems for plant. The most important problem related to the iron deficiency is the iron chlorosis. To the plants is essential that, in the soil, the reduction from Fe^{3+} to Fe^{2+} ion can occur, but the calcareous soils and carbonates presence reduce the possibility to realize this reduction [2, 3].

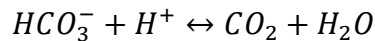
The $CaCO_3$ presence causes a sequence of reactions which make more alkaline the soil pH, increasing the iron deficiency of plant:



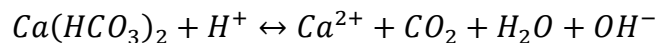
this salt in water is dissociated in calcium and bicarbonate ions:



the bicarbonate ion reacts with a proton giving carbon dioxide and water:



the protons consumption drives the reaction of water dissociation towards the release of protons and hydroxide ions, so the total reaction is:



From the total reaction is clear the formation of hydroxide ions with consequent growth of pH and with negative impact on iron availability in soil [4].

The others reasons can cause iron deficiency are clay soils, which limit the roots development and so the iron absorption, waterlogging which favors the pH enhancement and the presence of iron antagonist microelements (Mn, Cu, Zn, Co, Ni and Cd) with which competitive adsorption mechanism can occur [3].

1.1.2 The iron chlorosis

Iron (Fe) chlorosis is a widespread disease that affects the plants growth and reduces the yields of many crops. This nutritional disorder derives from iron deficiency and results in a decrease of chlorophyll content with a progressive yellowing of plants, starting from the younger leaves and proceeding through intervein till the older parts of plants. The causes of this complex problem are different, ranging from nutritional disorder to infections caused by microorganisms but the most important are the nature and the pH of soils, in fact the iron chlorosis is widely diffused in a calcareous and alkaline soils.

Because of diffusion of iron chlorosis various therapies have been developed and in particular the most diffused have been the use of iron salts and foliar spray. However both solutions have resulted not sufficient to treat this important disease because with the first one, the iron is readily converted in insoluble form, while with the second one only surfaces treatments can be done. Nowadays, the fertilization with synthetic chelates agents is the most common agricultural practice to solve the iron chlorosis and ethylenediamine-N,N'-bis(o-hydroxyphenyl) acetic acid (o,o-EDDHA) is among the most efficient iron chelating agents used [5]. The chelating agents are organic macromolecules which thanks to several functional groups are able to establish more than one chemical bond with the metal ion, stabilizing it and increasing its solubility.

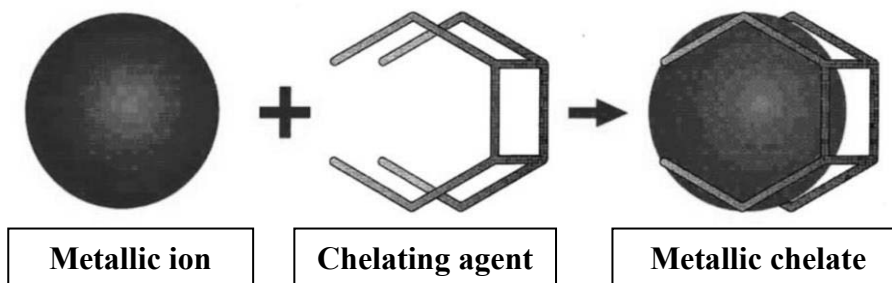


Figure 1. Metallic ion chelation sequence

In the iron chelates the chelating agent acts as carrier of iron ion (Fe^{3+}), carrying the ion up to the interface with the roots where it is released and subject to a reduction to Fe^{2+} after which it is absorbed by roots. The chelating agent, free of ion, returns in the solid phase of soil ready to trap the iron ions present in soil to carry to roots.

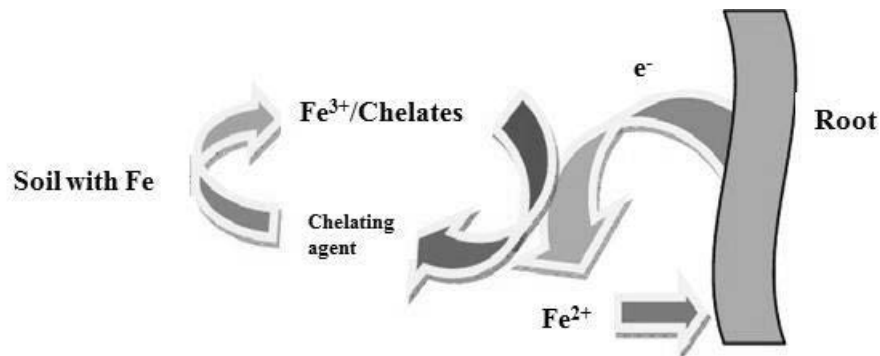


Figure 2. Iron absorption by plants

According to the agricultural practice the European commission, by the Regulation (EC) No. 2003/2003, regulates iron and other micronutrient chelates used as they are or incorporated in mixed fertilizers. Six chelating agents, all polyamine carboxylic acids, are permitted for this purpose: EDTA, DTPA, HEDTA, EDDHA, EDDH4MA and EDDCHA [6]. Ethylenediamine- N,N' -bis(*o*-hydroxyphenyl)acetic acid (*o,o*-EDDHA) and its analogues are the most important in the category of chelating agents [7].

The capability to complex the metallic ions is strictly dependent on chelating agent and soil characteristics, in particular the pH. For this reason the ethylenediamine- N,N' -bis(2-hydroxyphenylacetic acid), EDDHA, and in particular its chelated iron EDDHA/ Fe^{3+} is considered one of the best to treat the iron chlorosis. The isomer *o,o*-EDDHA/ Fe^{3+} is able to stabilize the iron ion and maintain it in solution thanks to the six bonds that the chelating agent can establish with the ion. For this reason this is the most diffused agent to solve the iron chlorosis [8].

However several studies have demonstrated that the industrial production processes of *o,o*-EDDHA/ Fe^{3+} produces a mixture of three regioisomeric products: *o,o*-EDDHA/ Fe^{3+} , *o,p*-EDDHA/ Fe^{3+} and *p,p*-EDDHA/ Fe^{3+} . The difference between the three isomers is in the number of bonds with which the chelating agent is bound with

metallic ion and in fact the *o,p*-EDDHA is able to establish five bonds with ion while *p,p*-EDDHA can offer only four bonds [9].

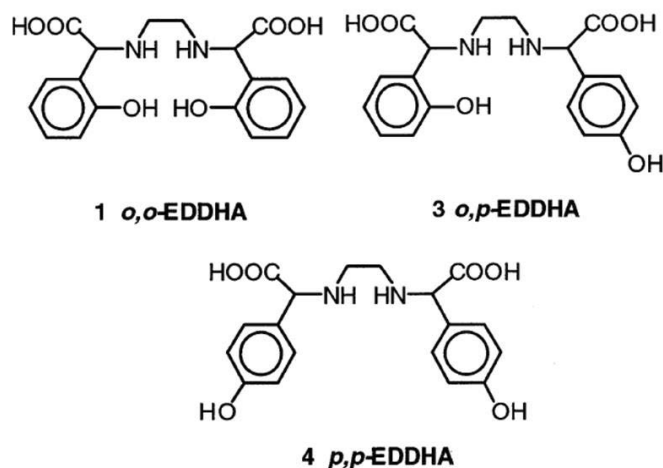


Figure 3. Structural formula of three positional isomers of EDDHA/Fe³⁺ [9]

The number of bonds with the metallic ion is the sign of stability and in fact the *o,o*-EDDHA/Fe³⁺ is the more stable of “family” and for this reason is used for a prolonged release, while the *o,p*-EDDHA/Fe³⁺ is used for the immediate treatment of iron chlorosis. In the industrial fertilizer a mixture of both isomers is present in order to guarantee the treatment of iron chlorosis from the beginning and for long time.

Furthermore the *o,o*-EDDHA/Fe³⁺ is characterized by two diastereoisomers: *meso-o,o*-EDDHA/Fe³⁺ and *rac-o,o*-EDDHA/Fe³⁺, stereoisomer which are not specular images between them and exhibit a different behavior [8].

1.2 State of art

The actual European law, UE rules n. 137/2011 of 16th February 2011, recognizes the iron chelates as water soluble products obtained by a chemical combination between the iron and one of authorized chelating agents. Moreover, in the European regulation are specified some important characteristics of product as the percentage of water soluble iron (5% w/w) of which the fraction of iron chelated shall be at least 80%, of which at least 50% chelated using the authorized chelating agents [10].

The European law obliges the producers to indicate the pH range to guarantee the product stability and the European standard for the analytical determinations.

Several production processes have been developed for the production of EDDHA chelated iron and, consequently, various studies have been carried out for the analytical detection of these compounds. To the complete knowledge about the iron chelated an overview of literature available about the o,o-EDDHA/Fe³⁺ production processes and its analytical detection is essential.

1.2.1 Synthesis processes

Many processes have been developed to produce o,o-EDDHA/Fe³⁺ drove by the necessity to obtain a cheaper and more pure as possible product and with a process simpler and advantageous as possible.

In the 1957 Kroll et al. [11], starting from the idea of Strecker [12], illustrated a process to produce iron chelates with a new chelating agent: the EDDHA. EDDHA was prepared by the addition of two moles of hydrogen cyanide to the Schiff base (obtained by the reaction from two moles of salicylaldehyde and one mole of ethylenediamine) to yield the dinitrile, which decomposed at 113-115°C. The dinitrile was hydrolyzed in concentrated hydrochloric acid at 40°C to the monoamide dihydrochloride. The EDDHA was obtained by refluxing the monoamide dihydrochloride in HCl and neutralizing the reaction mixture to pH 4, to precipitate the chelating agent. The EDDHA was purified by dissolving in dilute ammonia and reprecipitating at pH 4. The iron ions was added with EDDHA in water to form a deep red solution which in contrast with others metal chelates forms a stable compound [11].

Although this process ensures the production of very high purity EDDHA, it has never been used for industrial applications because of dangerousness and high price of hydrogen cyanide and the necessity to use expensive materials, able to be in contact with the aggressive substances involved in the chemical reactions.

In the 1958 Martin Dexter et al. [13] illustrated a new process to produce EDDHA without use the hydrogen cyanide. They proposed one step of reaction between sodium glyoxylate, ethylenediamine and phenol (or a substituted phenol) to yield a solution of desired product

according to the following reaction scheme wherein X may be hydrogen, halogen, sulfonic acid, carboxyl, hydroxyl or alkyl.

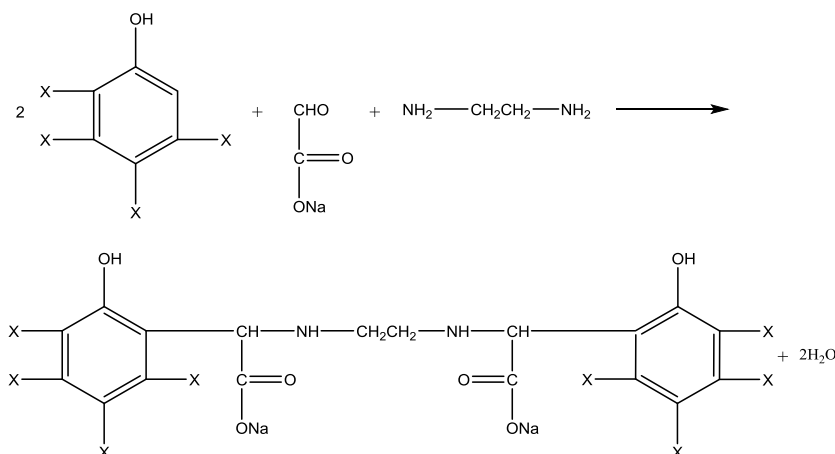


Figure 4. Reaction scheme of glyoxylate synthesis presented by Dexter

The reaction is preferred carried out at a temperature between 70°C and 100°C and at a pH between 8 and 10, preferably at 8.5. The phenol can be partially substituted with solvent such as methyl, ethyl or isopropyl alcohol. The chelating agent can be precipitated from solution by acidification of reaction mixture to a pH between 4 and 5. After removal of unreacted phenol by extraction or distillation, the EDDHA produced was chelated by the addition of concentrated water solution of iron salt.

Dexter showed some examples to produce EDDHA with a process based on the synthesis described and partially substituting the phenol in the reaction mixture, partially substituting the sodium glyoxylate and trying different reaction conditions.

This patent introduced the possibility to create a process potentially applicable in the industrial practice but some important problems were present in the described process. In fact, due to the low and variable reaction yield with the not still optimized reaction conditions, the severe reaction condition, 7 hours at a temperature between 70°C and 100°C, the strong variability of obtained products, this production process did not found an industrial application.

In the 1978 Petree et al. [14] patented a new process for preparation of phenolic ethylenediaminepolycarboxylic acid. They started from the consideration that Dexter US patent n. 2,824,128 [13] was a significant improvement respect the previous technology but they

noted as the formation of ortho-para isomer was predominant and, as explained before, the ortho-para isomer is less stable than desired ortho-ortho. This, based on the study of Kornblum, Bereigan and LeNoble [15, 16], was related to the solvent presence and with the reaction environment. They demonstrated, in fact, that the solvent in which the reaction is carried out can decide the course of reaction and in particular the dielectric constant and the solvating ions capacity drives the bond formation in ortho or para position on the carbon ring atoms.

Petree et al. observed that a variety of solvents have been tested to favour the ortho substitution on the phenol and with a non-aqueous solvents there were a lack of dissolving capacity while when water has been used as solvent, the product distribution was directed towards 80-90% of para undesired isomer.

The aim of Petree et al. was find a process to produce EDDHA ortho substituted with a reaction yield such as to justify an industrial scale up. He discovered that by using phenol both as reactant and as the solvent in the reaction system, the resulting phenol ethylenediaminepolycarboxylic acid product mix contains predominant amounts of the ortho-isomeric form (85% of the product mix is ortho-substituted). The free acid resulting can directly reacts with as iron salt to form the chelated iron, under alkaline conditions, The novel process follows the reaction scheme below:

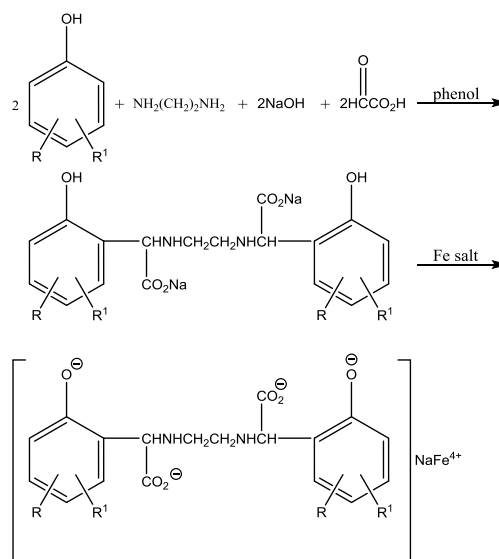


Figure 5. Petree reaction scheme

Wherein R and R¹ are independently selected from hydrogen, methyl, ethyl, halogen, methoxy, hydroxyl, carboxyl, sulfo and acetyl. Based on the above consideration, Petree and al. developed a process involved admixing molten or liquid phenol, ethylenediamine, glyoxylic acid and a base, that may be present in aqueous solution or selected from alkali metal hydroxides, bicarbonates and the like. The reaction proceeds at a temperature between 45-80°C for a period of about 2 to 16 hours, and preferably for 2 to 4 hours at 70-75°C. With respect to the molar ratios of phenol, ethylenediamine, base and glyoxylic acid Petree et al. suggest, respectively, range from 3 to 20, 0.5 to 0.55, 0.75 to 1.25, 0.95 to 1.25. The preferred molar ratio is 13:0.5:1:1 and it is possible to note that non-phenolic components are in stoichiometric quantity while the phenol is in large excess to accomplish its dual role.

At the end of reaction, by the action of an organic solvent and water is promoted the separation of phases present in the reactor where the water phase contains the EDDHA produced and the oil phase contains the excess of phenol and the organic solvent used for the separation. The water phase is ready for the chelation, at a pH between 7 and 8, with an iron salt to obtain the deep red, highly viscous, solution, which shall be dried to obtain the solid product. Typical iron salts for the chelation purpose include ferric sulfate, ferric chloride, ferrous chloride, and the like. The iron salts shall be used generally in excess molar amount of about 25%.

Petree et al. presented several examples of syntheses to validate their theory, reaching a reaction yield of about 60%, with the 5% of iron chelated of which at least 80% chelated in ortho-ortho isomers.

The Petree et al. patent of 1978 [14] represents a real milestone of industrial production of EDDHA/Fe³⁺ because they conceived a process with a good reaction yield and with a prevalence of desired product, using substances easier to manage respect the previous processes. The problems, nevertheless, are related to the very large use of organic solvents, which are absolutely not environmentally friendly substances, and the duration of process which is very long.

However, the process patented by Petree et al. in 1978 is nowadays the base of current industrial EDDHA/Fe³⁺ production.

In the 1989 Jullien [17] patented a new process starting from the consideration that the process invented by Petree [14] reached the goal

but using an enormous excess of phenol which after the reaction shall be eliminated from the product with a series of post processing operations and with a costs increase. The new process starts from the idea that the combined effect of an aromatic solvent and an appropriate catalyst can drives toward the same goal of Petree process but with a considerable reduction of phenol solvent and post processing operations.

Jullien proposed a reaction between phenol, glyoxylic acid, ethylenediamine, a base and a catalyst chooses between the oxides, hydroxides and the salts of zinc, magnesium, manganese, cobalt, iron, nickel, barium at al., in presence of an aromatic non polar solvent able to dissolve the reagents of reaction. The reaction was conducted at temperature between 60°C and 100°C; preferably between 85°C and 95°C, temperature range ensures the optimum reaction yield. The reagents was fed in stoichiometric quantity with a small phenol excess (2.5 times the stoichiometric). The catalyst, chooses preferably between oxides, hydroxides and salts of zinc, magnesium, manganese or molybdenum, was added in a very small quantity (0.001-0.05 moles of catalyst for each mole of glyoxylic acid). At the end of reaction, generally between 1 and 10 hours, the aromatic solvent can be separated from the water phase, containing the produced EDDHA, by the addition of water for simple decantation based on the different density. The extracted “water phase” can be directly used for the iron chelates formation. In fact, by the addition of an iron salt, under controlled pH condition that shall be maintained between 6.5 and 7, is possible produce the EDDHA/Fe³⁺ with a prevalence of ortho-ortho isomers. As iron salt Jullien suggests iron chloride, sulfates, nitrates or carbonates dosed with excess of 10-20% respect the stoichiometric. With the Jullien’s process is possible reach the 60% of yields in the desired product with about 80% of chelated iron in ortho-ortho isomers.

In the 2002 Sierra et al. [18] patented a process to produce the EDDHA based on the Kroll synthesis protocol. This process, that for above discussed problems did not found industrial application, guarantees very high purity product and satisfactory reaction yields. For this reason, the process described by Sierra et al. [18] is still used to the production of the o,o-EDDHA with a minimum purity of 98% used as standard for the analytical determination.

Regarding the industrial application, in the 2008 McKenzie et al. [19] patented a new process based on the idea to produce a plant fertilizer comprising the iron chelates, to nurse the iron chlorosis, and some others substances for the plant life and/or therapy of plant disease. In this patent McKenzie et al. also described the analytical method for EDDHA isomers detection but the most interesting part is the alternative production process proposed, compared with the US Patent 4,130,582 [14]. McKenzie “surprisingly” found that the feeding ratios described in Petree’s patent can be changed, with the result to produce a mixture of o,o-EDDHA/Fe³⁺ and o,p-EDDHA/Fe³⁺, using an excess of phenol between 2 and 5 time the stoichiometric and balancing the contents of water in the reaction mixture, in the range of 15% to 30% by weight of the reaction mixture, when reacts phenol with ethylenediamine and glyoxylic acid in presence of a base. Moreover, McKenzie has specified that when particularly low amounts of phenol are used, can be necessary the addition of an amount of inert organic solvent, allowing the reaction to run in a melt of phenol and to control adequately the reaction temperature between 40°C and 80°C. In this patent [19] the suggested reaction temperature is between 45°C and 85°C while the proper molar ratio of phenol, glyoxylic acid and a base respect to ethylenediamine are respectively 4 to 10: 0.5 to 0.55: 0.95 to 1.1: 0.75 to 1.25. The preferred bases are alkali metal hydroxides, in particular sodium hydroxide. Furthermore McKenzie et al. [20] investigated the role of reaction pH to convert the o,p-EDDHA into o,o-EDDHA, discovering as the low pH favors this conversion and in particular pH lower than 7 permits this conversion, at reaction temperature. This is an interesting observation which can permits to increase the ortho-ortho fraction when desired.

McKenzie et al. [19] in this patent have focalized the attention to the possibility to produce a mixture of ortho-ortho and ortho-para EDDHA ferric chelates which is not the crucial problem, considering that the current production processes already ensure the production of a mixture of isomers of the EDDHA. More interesting is the reduction of phenol use in the reaction feeding because it demonstrates the possibility to produce the ortho-ortho EDDHA also reducing the solvent excess.

1.2.2 Analytical determination

The analytical determinations are the most important methods to evaluate the quality of products present on the market, considering that the industrial processes are far to produce pure chelates products. As noted in the paragraph before, with all the described methods is possible produce a mixture of o,o-EDDHA/Fe³⁺, o,p-EDDHA/Fe³⁺ and p,p-EDDHA/Fe³⁺ and other polycondensation products only, and this makes essential the analytical determinations to know the characteristics of products.

Several methods have been proposed to determinate the chelated iron content:

- colorimetric methods [21], based on the measurement of the amount of colored light absorbed by a colored sample in reference to a colorless sample (blank). The spectrophotometer is an apparatus which allows the absorbance of a solution at a particular frequency (wavelength) of visual light to be determined. Colorimeter hence makes it possible to determinate the concentration of a known solute, since it is proportional to the absorbance;
- chromatographic methods [22], in which the mixture is dissolved in a fluid called mobile phase which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture move with different speeds and this cause a separation. This separation is based on the different partitioning between mobile and stationary phases. These methods allow to quantify the iron chelates present in the sample, permitting also to distinguish the isomers. After a comparative studies between colorimetric and chromatographic methods, the High Performance Liquid Chromatography (HPLC) have been found to be the best method to characterize the iron chelates.

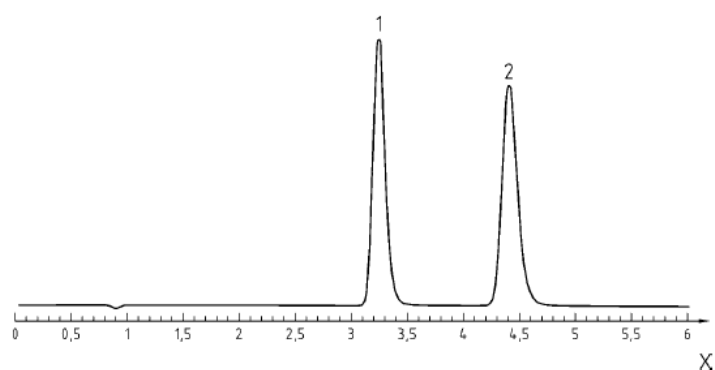
In the 2007 The European Committee for Standardization (CEN) released the European Standard EN 13368-2 (update of EN 13368-2:2001) [10] regarding the “determination of chelating agents in fertilizers by chromatography”. In particular, in the second part is present the “determination of Fe chelated by o,o-EDDHA and o,o-EDDHMA by ion pair chromatography”. The European standard is fundamental to find a common way to characterize the products in

order to avoid the existence of disputes about the chelated iron content.

The EN 13368-2:2007 specifies a method for the chromatographic determination of the iron chelated individual ortho-ortho isomer of the chelating agent o,o-EDDHA, allowing the determination of water soluble iron chelates but it does not allow the determination of free form of chelating agents. It is important note that with this method are considered both meso and dl-racemic isomers of the o,o-EDDHA/Fe³⁺.

According to this standard, the iron chelates are separated and determined by isocratic ion-pair high-performance liquid chromatography. When an iron chelate (anio) is added to a polar fluid (eluent), containing large cation, an ion pair is formed, which is retained by an apolar solid phase (stationary solid phase). The strength of the retention depends on the molecular size and its acidity. Then, each iron chelate exhibits a characteristics retention time, depending on the chelating agent, and it is separated from the other substances present in the sample. The separation is performed on a reverse phase silica column and using an aqueous solution of TBA⁺ (tetrabutylammonium) and acetonitrile as eluent. The detection is based on photometry at 280 nm. The standard imposes that all reagent shall be analytical grade and suggests that for the preparation of standard solution shall be used reagent with purity of 99% to avoid correction. The European standard imposes the equipment characteristics for the analytical determination and in particular the HPLC shall be equipped with an isocratic pump delivering the eluent at a flow rate of 1.5 ml/min, an injection valve with a 20 µl injection loop, a C-18 column with internal diameter 3.9 mm, a length 150 mm and dp = 5 µm and a UV/VIS detector with a 280 nm filter. The EN 13368-2:2007 also describes the preparation of eluent for the determination and Fe-o,o-EDDHA solution. The first one shall be prepared adding 20 ml of TBAOH (mass fraction 40% tetrabutylammonium hydroxide solution in water) to 600 ml of water and adjusting the pH to 6 with HCl. After the pH adjusting is necessary the addition of 300 ml of acetonitrile HPLC grade and the make up to 1 l volume with water. The eluent phase shall be filtered with 0.2 µm membrane. The Fe-o,o-EDDHA solution necessitates of some operations because are needed both the chelating solution and the EDDHA standard solution. To prepare the chelating solution is necessary to dissolve a ferric nitrate 9-hydrate (Fe(NO₃)₃·9H₂O) in

water reaching a concentration of $1\,050\text{ mg} \pm 30\text{ mg Fe/l}$. To prepare the standard solution, the European rule imposes to dissolve 0.322 g of o,o-EDDHA in 350 ml of water and 27 ml of NaOH solution. After the addition of 50 ml of chelating solution and five minutes of stirring, the prepared solution shall be stood over night in darkness, to allow the excess to precipitate as oxide. To calibrate the analytical method, from the starting standard solution some diluted solutions are obtained for dilution with NaOH buffer solution. From the analysis is obtained a chromatogram as below:

**Key**

- 1 dl-racemic Fe-o,o-EDDHA
- 2 meso Fe-o,o-EDDHA
- X time in minutes

Figure 6. Typical chromatogram of a 25 mg Fe/l of Fe-o,o-EDDHA standard solution [10]

The two peaks are representative of both ortho-ortho EDDHA isomers and measuring the peaks area is possible to calculate each isomer concentration. This chromatogram represents the reference for the analysis of commercial products that, obviously for the previous discussed reasons, include the others isomers, the unreacted reagent and some other polycondensation products.

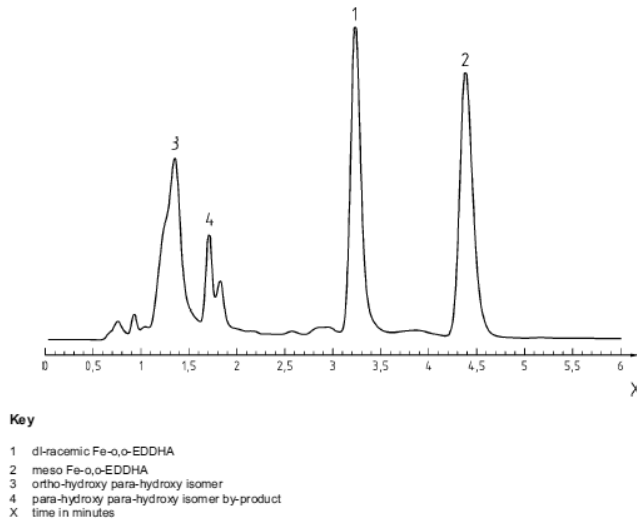


Figure 7. Typical chromatogram of a commercial Fe-o,o-EDDHA product [10]

The mass fraction w_{fe} of iron chelated by o,o-EDDHA in the fertilizer percent of sample is:

$$w_{fe} = \frac{c_{rac} + c_m}{m} \cdot 0.025 \quad [10]$$

where

c_{rac} and c_m are the iron concentrations of Fe chelated by racemic and meso isomers determined in milligrams per liter;

m is the mass of the sample considered for the analysis in grams.

The above discussed EN 13368-2:2007 o,o-EDDHA/Fe³⁺ detection method has been developed by Lucena et al. in the 1996 [23] and after it has been adopted as standard method by the European Community. As previously discussed the main product of iron chelates production is the ortho-ortho isomers but the production methods do not allow to produce “pure” o,o-EDDHA/Fe³⁺ but some others products are presents, in particular the o,p-EDDHA/Fe³⁺ isomers (p,p-EDDHA since two p-hydroxyphenyl groups are sterically impeded to blind Fe³⁺, does not form complex with Fe³⁺) [3, 6]. Due to its agronomic behavior the o,p-EDDHA has been recognized as chelating agent by the community law but no official detection methods have been developed to quantify its presence in commercial fertilizer [24]. In the 2006 Sonia Garcia-Marco at al. [24], with the support of EC, illustrated a method for the o,p-EDDHA/Fe³⁺ detection and quantification. They started from the consideration that the method

tuned for the o,o-EDDHA/Fe³⁺ is not suitable to determine the o,p-EDDHA/Fe³⁺ because it presents only five bonds between Fe and the chelating agent, respect the six bonds present in the ortho ortho isomers, and for this reason an optimum chromatographic elution is not achieved. For this main reason Sonia Garcia-Marco and her research group have developed the new detection method for o,p-EDDHA/Fe³⁺, however based on HPLC method. They started from the characterization of two standard ad hoc furnished by Syngenta Crop Protection in order to choose the best one to use for the experiment. After the preparation of o,p-EDDHA/Fe³⁺ with the adding of a water solution of Fe(NO₃)₃·9H₂O, to reach the final concentration of 1.54 mmol·l⁻¹, using an HPLC equipped with C-18 column with 5 μm particles, flow rate of 1.5 ml/min, injection volume of 20 μl and a detection wavelength of 480 nm, some tests to determinate the best composition of mobile phase have been performed.

They studied the effects of the pH, of ion pair reagent (TBA⁺) and organic modifier (acetonitrile). About the pH, they have maintained the percentage of acetonitrile and TBA⁺ concentration as described in the method elaborated by Lucena at al. [23] and tested some pH values (4, 5, 6, 7 and 7.75).

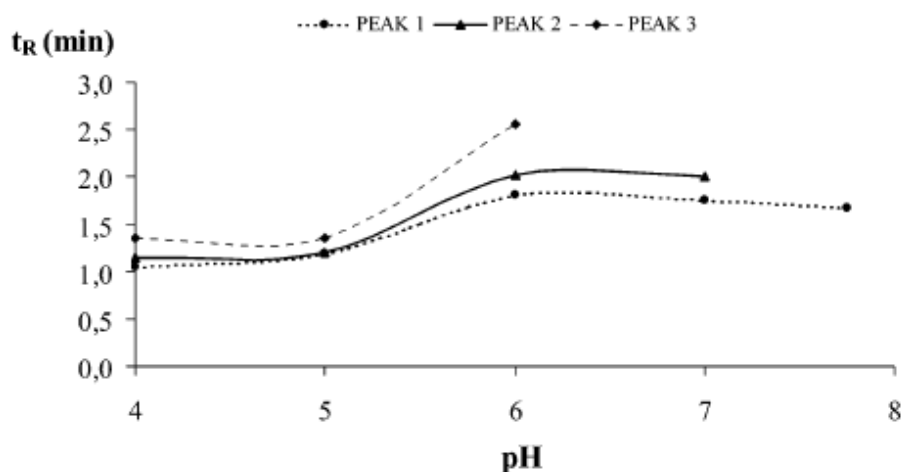


Figure 8. Influence of pH on the retention time of o,p-EDDHA/Fe³⁺ [5]

About the effects of TBA⁺ concentration, it has been investigated maintaining the percentage of acetonitrile (30%) and pH (both 5 and 7.75) constant and testing the TBA⁺ concentrations of 0.005, 0.01, 0.02 and 0.03 M.

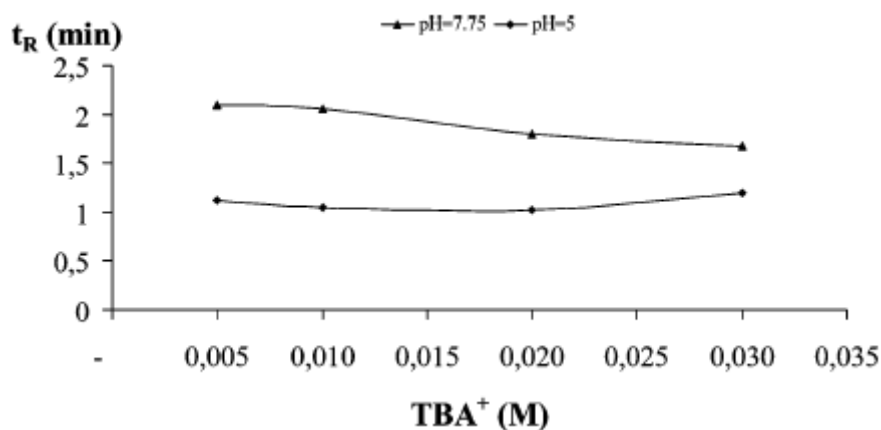


Figure 9. Influence of TBA⁺ concentration on the retention time of o,p-EDDHA/Fe³⁺ at two different pH values, 5 and 7.75 [24]

About the organic modifier, different percentages of acetonitrile in the eluent (20%, 25%, 28%, 30% and 35%) has been tested maintaining constant the pH and the TBA⁺ concentration.

About the pH has been possible to observe that the maximum retention time is near pH 6 and this value has been considered the most appropriate for the elution. Relating to the effect of ion pair reagent, at pH 7.75 the retention time decreases and as TBA⁺ concentration increased in the eluent, the chromatographic peak corresponding to the o,p-EDDHA/Fe³⁺ chelate become broader and a tail appeared. This fact made difficult its determination, and for this reason, a relatively low concentration of TBA⁺ was preferred. At pH 5 the retention time does not apparently change with the increasing of TBA⁺ concentration in the eluent. The t_R remains low at about 1.1 min. About the effect of organic modifier, the retention time of o,p-EDDHA/Fe³⁺ decreases as the acetonitrile content increases. In commercial sample both Fe³⁺ chelates, ortho ortho and ortho para isomers, are present and the presence of an organic modifier is necessary to change the polarity in the eluent and compete with TBA⁺ in the adsorption equilibria to control the elution rate. Low percentage of acetonitrile increases (until 30 minutes) the retention time of o,o-EDDHA/Fe³⁺ and the use of 20% and 25% percentage avoids the isocratic separation. To reach the isocratic separation has been chosen an high acetonitrile concentration (35%). According to the studies discussed above, Sonia Garcia-Marco et al. created [24] a chromatographic method for the determination of o,p-EDDHA/Fe³⁺ using a mobile phase made up of 35% acetonitrile in a 0.005 M TBA⁺

aqueous solution at pH 6 and calibrated using the o,p-EDDHA standard, obtaining a linear calibration for o,p-EDDHA/Fe³⁺.

All the research work of Sonia Garcia-Marco and her group was aimed to the analysis of commercial chelates and in fact they shows the results of analysis of o,o-EDDHA/Fe³⁺, of o,p-EDDHA/Fe³⁺ and of a commercial product (Figure 10).

The chromatogram of commercial product shows three peaks at 1.15, 2.11 and 2.60 min (t_R) and comparing this with the chromatogram of standards chelates, the first peak can be assigned to o,p-EDDHA/Fe³⁺ and the next two peaks are the two diastereoisomers (racemic and meso, respectively) of o,o-EDDHA/Fe³⁺.

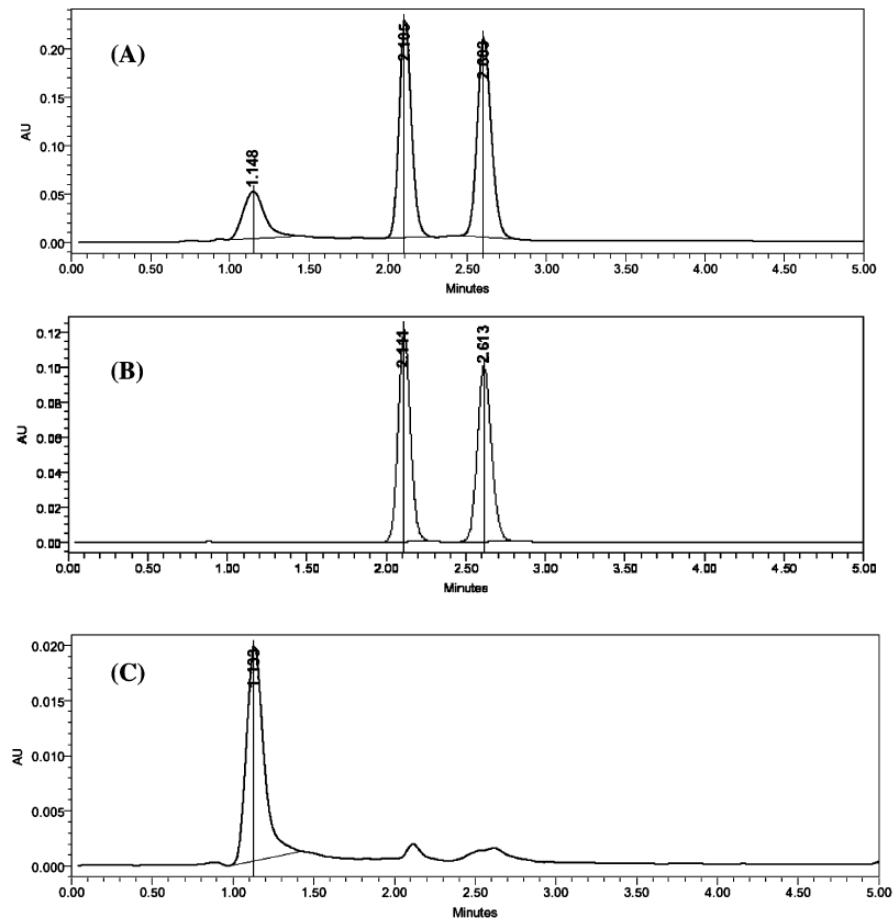


Figure 10. Chromatograms and UV-VIS spectra at 480 nm for (A) a commercial EDDHA/Fe³⁺ fertilizer, (B) an o,o-EDDHA/Fe³⁺ standard and (C) an o,p-EDDHA/Fe³⁺ standard [24]

The chromatogram of commercial product proposed by Sonia Garcia-Marco is very clean and in fact they have explained that in the four commercial samples analyzed they found some other peaks, the clear demonstration that during the synthesis of EDDHA/Fe³⁺ some other products are obtained as well as the ortho-ortho and ortho-para isomers. Several scientists have worked and still work on the exact determination and quantification of undesired product of o,o-EDDHA/Fe³⁺.

In the 2001 Cremonini et al. [25] have analyzed by Nuclear Magnetic Resonance (NMR) a commercial samples of EDDHA/Fe³⁺ to confirm as discovered by HPLC analysis Hernandez-Apaolaza et al. in 1996 [26]. Both H NMR and C NMR show more peaks than expected and this can be reasonable explained by the presence of other positional isomers of o,o-EDDHA/Fe³⁺.

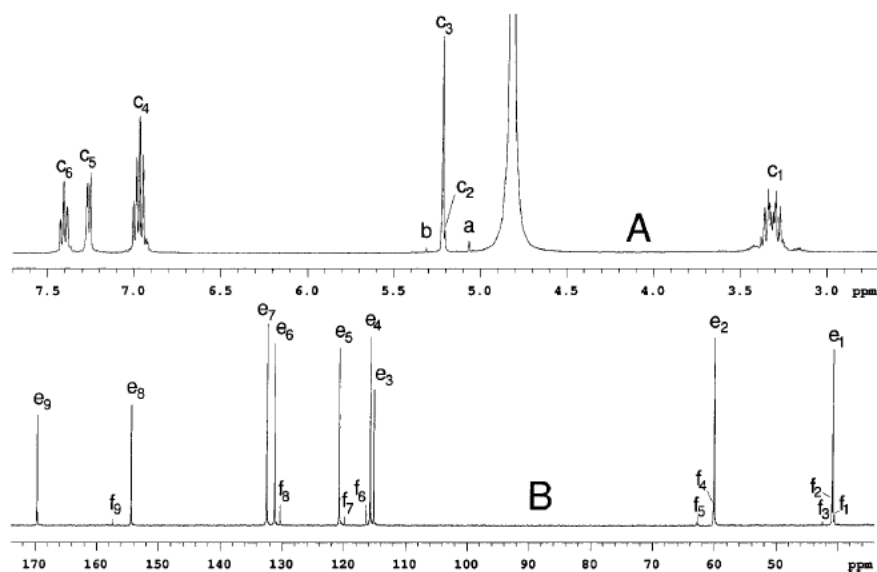


Figure 11. NMR spectra of a commercial sample of EDDHA in D₂O at 308 K (400 MHz): (A) ¹H spectrum; (B) ¹³C spectrum

As possible to note by the spectra A five peaks appear (c₁, c₂, c₃, c₄, c₅) can be assigned respectively to aliphatic CH₂'s, benzylic methenes of the two diastereoisomers and four aromatic protons. Two peaks more (a and b) indicate the presence of impurities. In the spectra B this explanation is confirmed by the presence of some peaks more than expected (all f peaks). The presence of different products from

o,o -EDDHA/ Fe^{3+} is completely confirmed by the further analysis made by Cremonini et al. [25].

Clarified the production of others product besides o,o -EDDHA/ Fe^{3+} by the synthesis of iron chelates, it is important to understand the effects of these compounds on the characteristics of fertilizers.

In the 2006 Lourdes Hernandez-Apaolaza et al. [27] have published a research regarding the structure and the properties of co-products formed during the EDDHA synthesis. In this paper they started from the awareness of production of o,p -EDDHA/ Fe^{3+} isomers during the synthesis of EDDHA chelated iron [9, 28], but they have considered that fertilizers also contain variable extra amounts of soluble iron did not corresponding to any chelates. Additional studies have demonstrated that byproducts present in commercial fertilizers could form 2:1 [Fe^{3+} /ligand] complexes; although their nature and their ability to transfer iron to the plant was still unknown [25]. Lourdes Hernandez-Apaolaza et al. have analyzed the structure of these compounds and their effects as substrates of the enzyme ferric reductase (FCR) in a cucumber plant and their efficiency to provide iron to soybean plants [27]. They produced the EDDHA/ Fe^{3+} according to a glyoxylat synthesis and by HPLC was determined the produced chelates. By High Performance Liquid Chromatography is possible to detect the substances present in the sample but for a best understanding of nature and characteristics of byproducts is better the applying of electrospray ionization mass spectrometry (ESI-MS). By the analyses and by the applying of produced chelates in the cucumber treatment they found that the synthesis of commercial EDDHA yields a mixture of o,o -EDDHA, o,p -EDDHA, p,p -EDDHA and other byproducts also able to chelate iron, but these oligomeric EDDHA-like compounds have a limited value as iron fertilizers. They do not have the long-lasting effects of o,o -EDDHA/ Fe^{3+} not the fast action of o,p -EDDHA/ Fe^{3+} and for these reason their fertilizing effects is reduced.

Chapter Two

Aims of the work

2.1 Aims of work

Although the consumption of iron chelates for agricultural uses would think of a strongly consolidated area, in the field of the production of chelated iron there are several research spaces.

The research work has been focused to the complete knowledge of the production process of o,o-EDDHA/Fe³⁺, through the realization of a wide experimental work, aimed to the development of a more efficient and more sustainable production process.

In order to reach the aims, starting from the current reference industrial process, the work has been focused to the determination of reaction kinetic, necessary analysis to optimize the reaction time, to the elimination of hazardous substances involved in the process and to the reduction, as much as possible, of the use of the organic solvents. This has been a crucial goal and to develop an environmental sustainable process, the recycle in a closed loop, of the organic solvents fed in the process, has been tested.

The research work has been supported by a company interested to the development of an industrial applicable process and, based on the results of research the design of an industrial plant has been performed and the supervision of the industrial plant construction has been supplied.

By virtue of this, the developed process has been industrialized and the developed product will be available on the worldwide market.

Chapter Three

Materials and methods

3.1 Materials

The materials used and the products obtained during the experimental work are following presented.

3.1.1 Reagents

The reagents used has been supplied by Sigma Aldrich, Milan, Italy and following are shortly described the main characteristics.

Phenol

The phenol (CAS n° 108-95-2), also known as carbolic acid, is an aromatic organic compound with the molecular formula C_6H_5OH . The molecule consists of a phenyl group ($-C_6H_5$) bonded to a hydroxyl group ($-OH$), which at room temperature is solid in form of needle-shaped crystals. The aromatic ring ensures a good solvent capacity to dissolve the organic reagent and for this reason, in our scope, it is used both as reagent and as solvent. In the synthesis of the EDDHA participate two phenol molecules that they furnish two of six bonds with which the metallic ions is linked.

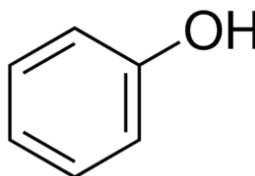


Figure 12. Phenol chemical structural formula

Ethylenediamine

The ethylenediamine (CAS n° 107-15-3) is an organic compound with the formula $C_2H_4(NH_2)_2$. At room temperature is colorless liquid (lightly yellowish), very volatile. The presence of two functional groups $-NH_2$, with two free electrons on nitrogen atom, gives high reactivity and permits the formation of “bridge” between the two phenolic groups of EDDHA. Moreover, the not shared electrons present on the nitrogen are linked to the metallic ion, occupying two of remaining four free bonds.

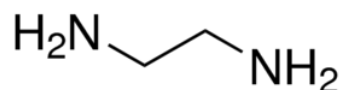


Figure 13. Ethylenediamine chemical structural formula

Sodium Hydroxide

The sodium hydroxide (CAS n° 1310-73-2), NaOH, also known with the commercial name of caustic soda, is a strong chemical base available in form of white solid pellets. During the experimental work it has been used caustic soda water solution 50% w/w, colorless and odorless liquid very corrosive, and mild sodium hydroxide solution, obtained dissolving one pellets of solid caustic soda in 1 l of distilled water.

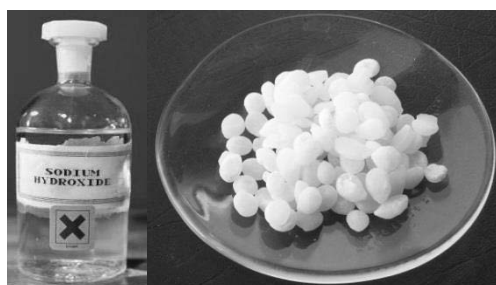


Figure 14. Liquid (on the left) and solid sodium hydroxide

Glyoxylic acid

The glyoxylic acid (CAS n° 563-96-2), molecular formula $\text{C}_2\text{H}_2\text{O}_3$, is an organic compound of family of carboxylic acids. It has been used as water solution 50% w/w and it appears as colorless liquid, lightly yellowish. The glyoxylic acid furnishes the last couple of bonds with which the chelating agent links the metallic ion, permitting the reaction to start.

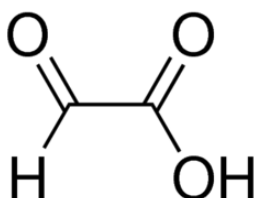


Figure 15. Glyoxylic acid chemical structural formula

Toluene

The toluene (CAS n° 108-88-3), chemical formula C_7H_8 , is a water insoluble organic solvent. It is constituted by an aromatic ring where one hydrogen atom is substituted with a $-CH_3$ group which ensures the solvent capacity. Toluene at room temperature is a colorless very volatile liquid. It is fed with the others reagents with solvent function only, without participating at the reaction but contributing to a best mix of reagents.

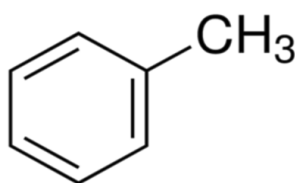


Figure 16. Toluene chemical structural formula

2.1.2 Solvents

As solvents during the EDDHA reaction production have been used phenol and toluene, of which the characteristics which have been previous described.

About the solvents used to prepare the eluent solution of mobile phase for the HPLC determinations, acetonitrile and tetrabutylammonium hydroxide have been used.

Acetonitrile

The acetonitrile (CAS n° 75-05-8) is a chemical compound with the formula CH_3CN where a nitrile is bonded with a methylic group. It is a colorless liquid, used as component of mobile phase for HPLC analysis.

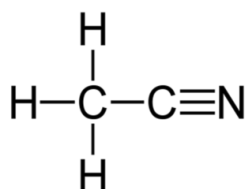


Figure 17. Acetonitrile chemical structural formula

Tetrabutylammonium hydroxide

The tetrabutylammonium hydroxide (CAS n° 2052-49-5) is a chemical compound with the formula $(C_4H_9)_4NOH$ and with acronym TBAOH or TBAH. This substance is not readily obtainable as a pure compound, but it is employed as solution in water or alcohols. The tetrabutylammonium hydroxide has been used with acetonitrile and water as component of eluent for the HPLC analysis.

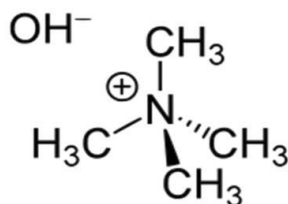


Figure 18. Tetrabutylammonium hydroxide chemical structural formula

*2.1.3 Iron salt**Iron(III) nitrate nonahydrate*

The iron(III) nitrate nonahydrate (CAS n° 7782-61-8) is the iron salt of nitric acid. This chemical compound, with formula $Fe(NO_3)_3$, is a deliquescent and for this reason it is found in its nonahydrate form $Fe(NO_3)_3 \cdot 9H_2O$ in which it forms a colorless to pale violet crystal.

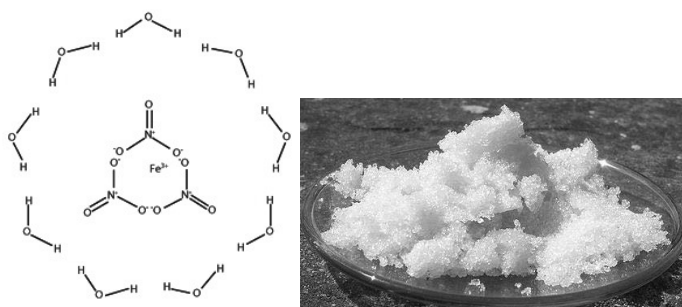


Figure 19. Chemical structure of Iron(III) nitrate nonahydrate (on the left) and a picture of iron salt

Iron(III) sulfate

The iron(III) sulfate, known as ferric sulfate (CAS n° 15244-10-7), is the sulfate of trivalent iron ion. The chemical compound, with formula

$\text{Fe}_2(\text{SO})_4$, is a rhombic crystalline, usually yellow, soluble in water at room temperature

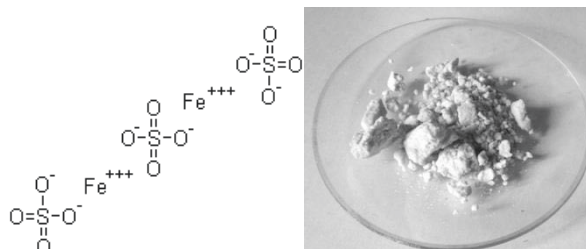


Figure 20. Chemical structure of Iron(III) sulfate (on the left) and a picture of iron salt

The iron (III) sulfate supplied by Sigma Aldrich has an hydration rate of about 1.8% w/w, with a molar ratio water/anhydrous salt of about 40%. Based on these information, it has been possible to calculate the molecular weight of iron (III) sulfate equal to $407.332 \frac{\text{g}}{\text{mol}}$.

2.1.4 Product: *o,o*-EDDHA/ Fe^{3+}

The synthesis reaction permits to produce the chelating agent EDDHA, the ethylenediamine- N,N' -bis(2-hydroxyphenylacetic acid), with chemical formula $\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_6$, which in the second phase, by the addition of iron(III) salt, leads to the production of its chelated iron: EDDHA/ Fe .

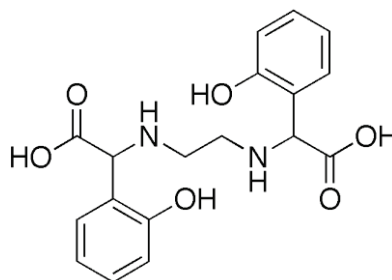


Figure 21. EDDHA molecular formula

The EDDHA can establish till six bonds with the iron ion, depending of the isomeric form of chelated iron: *o,o*-EDDHA/ Fe^{3+} , *o,p*-EDDHA/ Fe^{3+} and *p,p*-EDDHA/ Fe^{3+} .

The *o,o*-EDDHA is the isomer with higher surplus value because is the more stable; it is characterized by two diastereoisomers, meso and dl-racemic form.

2.2 Equipment

2.2.1 Pilot plant

The experimental work has been carried out using a pilot plant designed, realized and managed by the research group.

Two batch glass reactors, each 2 liters volume, have been used. Each reactor is equipped with a glass jacket for the fluid circulation, necessary to regulate the temperature of reacting mixture. The jacket of the first reactor, used for the EDDHA synthesis, is connected by two silicic pipe, one to the inlet of jacket and the other one to the outlet, to the managed by a computer thermal bath. In the second reactor, necessary for the chelation of produced EDDHA, the jacket is not connected with a thermal bath because the chelation reaction is carried out at room temperature. Both reactors are covered with a lid provided with four necks necessary to guarantee the reagents inlet, the installation of pH probe and temperature thermocouple, the installation and the rotation of agitator shaft and pipes inlet for acid and base. In order to guarantee a perfect mixing of reacting mixture both reactors are equipped with an agitator provided with a variable speed motor and switch on/off. Moreover, based on the different characteristics of reaction mixture, liquid the EDDHA synthesis reaction environment and doughy highly viscous the chelation reaction mixture, the shaft and helix of agitators are different and able to support the mechanical stresses generate during the mixing phase. On the bottom of each reactor a valve to empty the reactors and to facilitate the cleaning is placed.

Each reactor is equipped with a pH probe and temperature thermocouple connected with acquisition data board managed by computer. The temperature is measured by OMEGA thermocouple model CPSS-IM15U-300, T type thermocouple (Copper-Constantan with stainless steel sheath) with a measurement range $-270^{\circ}\text{C} \div 400^{\circ}\text{C}$. The pH is measured by OMEGA pH probe model PHE-5321-10, an instrument suitable for harsh industrial environment with a non-fouling annular ceramic junction and electrode housing of PVDF with 316 SS locking pins. Dual "O" ring (1 EPR and 1 KFM) are supplied to prevent process contact with potting epoxy. The pH sensor used is characterized by a measurement range between 0 and 13 with a temperature range $-5^{\circ}\text{C} \div 110^{\circ}\text{C}$. The pH probe has been calibrated by

using three buffer solution at known pH and measuring the relative tension:

Table 1. Voltage related to pH of buffer solutions

Tension [mV]	pH
0	7.29
0.24	3.06
-0.15	10.01

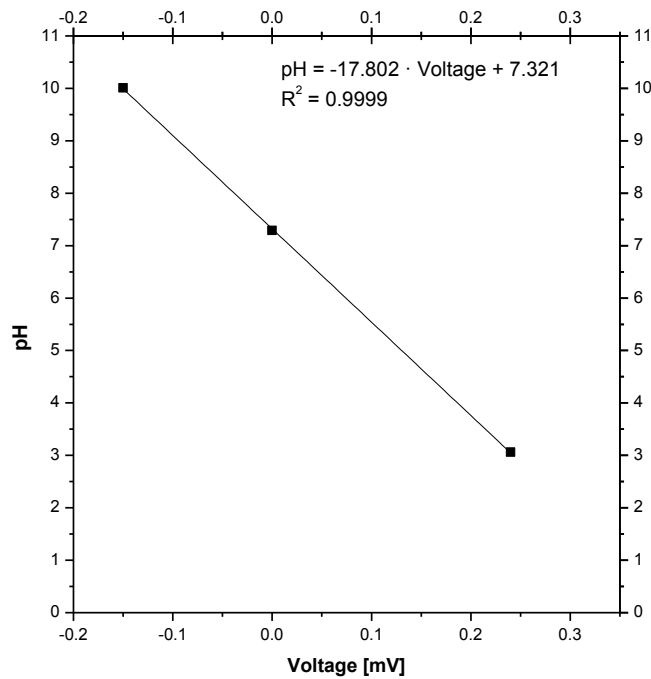


Figure 22. Voltage vs. pH trend for the buffer solutions used to calibrate the pHmeters

The data have been fitted by a linear fitting and it has been found a linear law between pH and signal:

$$y = -17.802x + 7.321$$

Each instrument, pHmeter and temperature thermocouple, is equipped with a BNC connector and is connected with an acquisition data board. The acquisition board is connected by USB to the personal computer and this permits to manage the plant by using computer's software.

The plant is managed by two software: Julabo and an ad hoc program wrote in LabVIEW. The first one ensures the management of thermal bath and by the selection of desired temperature or of desired thermal cycle, it permits to guarantee the thermic history necessary to the correct development of the EDDHA production reaction.

The second one is an ad hoc program wrote in LabVIEW which permits to record the thermal history developed in the reactors, with the temperature measured by thermocouple, and the pH history occurred in the reactors. Moreover, the program permits to decide the time of data sampling but the most important function of program is the control and the automatic adjustment of pH. During the chelation reaction, in fact, is fundamental that the pH is comprised between 7 and 8 and the best solution to ensure the achievement of this target is the application of feed-forward control system. By the program is possible to set the set point pH value shall be reached in the second reactor and by the measuring of the pH probe, inserted in the second reactor, is possible to compare the set point value (7.5 pH) with the measured pH. The plant is equipped with two peristaltic pumps, one for the dosing of NaOH water solution 50% w/w and the other one for the HCl 37% water solution w/w, which are automatically activated by the program comparing the pH measured with the set point value; if the measured pH is more acid than set point caustic soda is added, if recorded pH is more alkali than set point hydrochloric acid is dosed in order to reach the set point value. However, because to the EDDHA water solution is added, during the chelation, an iron salt, the pH goes immediately down and is necessary the addition of a base to reach the desired pH.

The peristaltic pump is a positive displacement pump equipped with a flexible pipe fitted inside a circular casing of pump. The rotor of pump, equipped with a number of rollers, compresses the flexible pipe and as the rotor turns a part of pipe under compression is pinched closed thus forcing the fluid to be pumped to move through the tube. Moreover, as tube opens to its natural state after the passing of cam, fluid flow is induced to the pump. This process is called peristalsis and for this reason this kind of pump is called peristaltic pump.

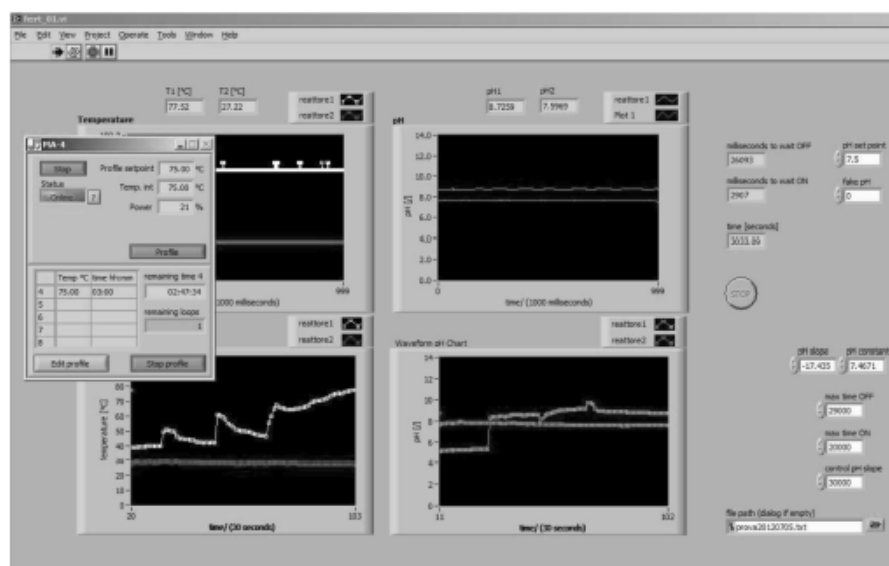


Figure 23. Graphic interfaces of software used

2.2.2 High Performance Liquid Chromatography

The High Performance Liquid Chromatography, better known with its acronym HPLC, is a chromatographic technique used to separate the components in the mixture, to identify and quantify each component. This analytical technique can be described as a mass transfer process involving adsorption where it is used the affinity equilibrium between a “stationary phase”, placed inside the column, and a “mobile phase”, that it flows through the column. The active component of the column, the sorbent, is typically a granular material made of solid particles ($2 \div 50 \mu\text{m}$) in order to create a big specific area available to adsorption, and the interaction degree between the substance present in the mobile phase and the stationary phase is the cause of mobile phase separation in the several constitutional compounds. This means that if in mobile phase is present a substance with low affinity with stationary phase, they will establish weak bond and the time to pass the stationary phase is low; on the contrary if there is affinity between compound of mobile phase and stationary phase, they will establish strong interaction and more time is necessary to pass the stationary phase. The time necessary to the substance to pass through the columns is defined retention time. More is the affinity between mobile phase and stationary phase and longer is the retention time.

Because of the size distribution of stationary phase is very low, it is necessary to pump the mobile phase, where is dissolved the compound of interest, through the column and during the analysis high pressure, of order of hundred atmosphere, are reached.

The characteristics of stationary phase are the hydrolysis and high temperature stability and high pressure mechanical resistance. The column shall be realized in high pressure resistant material, generally stainless steel, and it shall be insert in a jacket where it will be applied a pressure such to equilibrate the pressure of mobile phase in the column.



Figure 24. HPLC apparatus used

The HPLC apparatus used is the Agilent Technology model 1260 Infinity with the column of Agilent model ZORBAX Eclipse Plus C18, 4.6 mm x 150 mm of dimensions and porosity of 3.5 μm . The eluent solution is realized using as solvent distilled water HPLC grade, acetonitrile and TBA⁺.

In order to prevent stationary phase damages due to the size of particles contained in the mobile phase, a pre column, upstream of column, is inserted to filter the solution inlet to the column. The mobile phase shall be always filtered before used but despite this a pre filtration of sample to be analyzed is necessary.

The sample is taken by a needle and by pump is pumped, with the mobile phase, through the column. The flow rate is regulated by a flowmeter according to the set parameters. At the outlet of

chromatographic column is placed an UV VIS lamp to detect the outlet substances and each time which is observed a passage, a peak, function of concentration, on the chromatogram is marked.

From the analysis is obtained a chromatogram where is reported the trend of absorbance versus the elution time under the regulation of Lambert & Beer law for the diluted solution. The Lambert & Beer law describes the electromagnetic radiation adsorption phenomena and is the milestone of spectrophotometry. Considering a light fraction of I_0 intensity at a certain wavelength, in our case 280 nm, passes through a solution contained in a cuvette and I is the intensity of light out to the cuvette, is possible define the adsorbed light by sample as I_0-I .

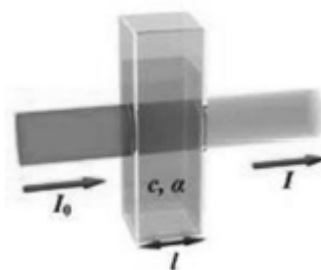


Figure 25. Sample of l thickness, c concentration and α diffusivity

It is possible define the absorbance as:

$$A = \log_{10} \frac{I_0}{I} = \log_{10} \frac{1}{T} = \epsilon l C = k C$$

The absorbance (A) is defined as the logarithm of inverse ratio of transmittance (T) or as the product of specific molar absorbance (ϵ) of optical path (l) and of concentration (C) of solution contained in the cuvette. The proportional constant k is a product of optical path (l) and specific molar absorbance (ϵ) and it considered constant for each substance at a certain wavelength although is a weak temperature function.

The Lambert and Beer law is applicable for dilute solution and it relates the light adsorbed by a substance with its concentration, its chemical characteristic and with the thickness of crossed medium.

2.3 o,o-EDDHA/Fe³⁺ synthesis

The experimental phase is based on the production of o,o-EDDHA/Fe³⁺ and the sequence of operation necessary to reach this goal is following described.

2.3.1 The first reaction phase: EDDHA production

The production process starts with the synthesis of the chelating agent. After the preparation of reagents, two steps follow: the first step of feeding and conditioning of reagents and the second step of very reaction phase, respectively of 30 minutes and 3 hours.

The feeding of reagents is divided in several phases, depending of type of reaction performed, and these will be described in the next chapter where each synthesis will be specifically explained. However, the synthesis reaction occurs in the first reactor and the reagents are fed manually through a dedicated neck in the lid. During the feeding the internal reaction temperature is maintained, using the thermal bath, at $45^{\circ}\text{C}\pm 2$ in order to promote reagents mixing and to condition the reagents.

Ended the fed reagents phase, the inner reaction temperature is increased till $75\pm 5^{\circ}\text{C}$, the temperature necessary to the correct realization of reaction. The reaction time suggested by literature is 2/3 hours and at first stages this time has been considered.

In order to obtain information about the reaction evolution, every 10 minutes a sample from reacting mixture has been taken (2 ml of reaction mixture) and analyzed by HPLC.

2.3.2 Phases extraction

At the end of reaction time, the reactor is cooled down at $20^{\circ}\text{C}\pm 5$ by the circulation of cold water in the jacket of reactor and by the adding of solvents. This phase is important because with low temperature the reaction is stopped and the extraction of phases can be performed.

To promote the separation of phases present in the reactor, water and not water miscible solvent (toluene) are introduced in the reactor by the neck on the lid. The separation occurs due to the different density of water phase respect oil phase, containing the toluene and unreacted phenol, and for their not miscibility.

After few minutes the water and oil phases are clearly separated, with the oil phase, composed by unreacted phenol and toluene and lighter than water compounds, in the upper layer and water phase, containing the produced EDDHA and the unreacted reagents, in the lower layer.

By the valve on the bottom of reactor both phases are collected and the water phase is fed in the second reactor.

The same proportion water-toluene, used to facilitate the phases separation in the reactor, has been used for the samples taken during the reaction to analyze the kinetics aspects. In fact, related to the withdrawn volume sample, a proportional quantity of solvents are previously inserted in a test tube placed in a fridge, in order to block immediately the development of reaction at the moment of sampling.

2.3.3 The second reaction phase: the chelation

The second reaction phase is realized in the second reactor by the addition to the water phase extracted from first reactor, containing the produced EDDHA, of a concentrated solution of iron salt. In order to ensure the ortho-ortho substitution of EDDHA is important the perfect stirring of reaction mixture and it is essential that during the chelation the pH shall be absolutely maintained between 7 and 8 pH, as indicated by literature.

This regulation is realized by the dosing of NaOH water solution 50% w/w or hydrochloric acid water solution 37% w/w with the peristaltic pumps managed by a software. The collection of data time and the off time of survey by the operator and these command the start/stop and the action time of pumps.

Since the addition of iron salt causes a marked acidification of reaction mixture, a caustic soda water solution 50% w/w is manually introduced immediately after the iron salt addition, in order to faster reach the necessary pH and increase quickly its value around 7.5 and by the pumps the pH is adjusted at the set point value.

The chelation reaction proceeds till the stable achievement of desired pH and at the end of reaction the product is extracted by the bottom valve and is collected in a flask, ready to the drying.

The chelation is also performed on the water phase extracted from the samples taken during the first reaction phase, by the addition, in proportional amount, of the chelating solution at the same iron concentration of that used for chelation of produced batch. The pH is monitored by the pHmeter and the adjusted around 7.5 by the use of HCl 37% water solution w/w and NaOH 50% water solution w/w.

2.3.4 Drying

The product extracted from the second reactor, containing the o,o-EDDHA/Fe³⁺, is a highly viscous liquid mixture and in order to end the production process is placed in aluminum box and dried in oven at 80°C ±5.

The dried product is quantitatively removed from the box and grinded in order to obtain the characteristics water soluble red/brown powder.

The same process, but with a smaller quantity, is performed on the samples extracted during the reaction.



Figure 26. Dried samples extracted during synthesis reaction (on the top) and dried final product (on the bottom)

2.3.5 Plant washing

At the end of each synthesis both reactors shall be carefully cleaned to remove the remaining chemicals and products and to prepare the plant to the next batches.

The washing is realized by using water and an adequate cleaning agent. At the same time, the instruments present in the reactor shall be cleaned to avoid incrustations and subsequently damages.

2.4 Reaction scheme

To develop the research work and to reach the aims, some reaction schemes have been tested, starting from processes presented in the literature and proceeding with progressive modifications and optimizations.

2.4.1 Basic reaction scheme

The reaction scheme chooses as starting point of experimental work is the Petree et al. patent of 1978 [29], which is the most important process used in the industrial practice. As above described, this process necessitates of a very large phenol excess which it acts as reagent and as solvent, favoring the mixing of reagents.

The proposed chemical reaction is the following:



with a stoichiometric molar ratio respectively of **1:0.5:1:1**.

The reaction occurs according to the follow reaction path:

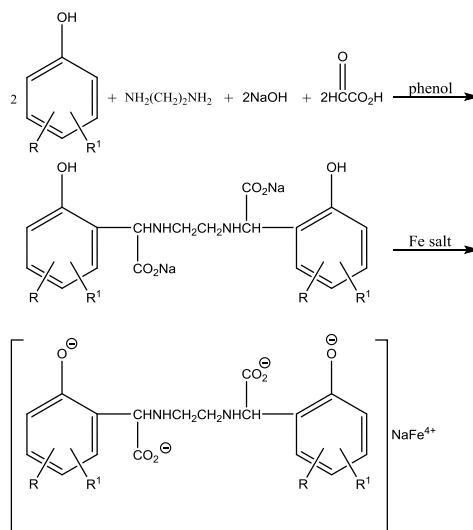


Figure 27. Reaction scheme of Petree et al patent

and the suggested fed molar ratio is:



in which is possible to note the phenol excess of 12 times the stoichiometric.

The reaction has been realized according the described patent conditions but with some technical modifications necessary for the research scopes.

According to the first aim of work, all the chlorinated substances used in the process described by the patent have been replaced and in particular, the extraction of phases has been realized by the three washing with solvent, as suggested by patent, but substituting the carbon tetrachloride with the toluene and as iron salt has been used the iron (III) nitrate nonahydrate instead of iron chloride.

The production process starts with the feeding of reactants and according to the sequence suggested by the Petree et al. [29] patent the phenol, solid at room temperature, has been melted and introduced in the reactor. After 10 minutes the ethylenediamine has been added and after other 10 minutes the NaOH 50% water solution w/w has been dosed. After 10 minutes, the glyoxylic acid 50% w/w has been introduced and the reactor temperature has been increased from $45^{\circ}\text{C}\pm 2$ to $75\pm 5^{\circ}\text{C}$ and the reaction it was going on for three hours.

To check the reaction evolution, from the starting time, has been taken 2 ml samples of reaction mixture each 10 minutes; only after the first 5 minutes of reaction a sample of 2 ml has been taken to zoom the first reaction stage.

At the end of reaction time, the extraction of two phases of reaction mixture has been realized in the first reactor by the three washing with water and toluene.

The separation of phases present in the samples taken during the reaction has been realized using 3.6 ml of water and 4 ml toluene for each sample, respecting the molar ratios sample/water and sample/toluene adopted for the extraction of phases in the reactor.

The water phase obtained from the extraction phases has been transferred to the chelation reactor where, under controlled mixing conditions, a concentrated solution of iron (III) nitrate nonahydrate has been added. The pH has been rapidly increase in the range of set point value, by the manual adding of caustic soda 50% water solution w/w, and stabilized at the 7.5 pH value set point by the use of acid and alkali solution dosed by the peristaltic pumps.

The product obtained has been extracted from the reactor by the bottom valve and placed in oven at $80^{\circ}\text{C}\pm 2$. After the drying the

product has been recovered scratching the box and it has been grinded to obtain the typical red-brown powder.

2.5 Analytical determination

The analytical determination has been carried out according to UNI EN 13368-2 related to the determination of chelating agents and iron chelates with o,o-EDDHA in fertilizer with High Liquid Performance Chromatography. In this work has been analyzed both the final product and the chelated samples extracted during the first reaction stage.

2.5.1 HPLC analysis

The first operation is the calibration of the analytical method and this is realized by the chelation with an iron salt of a standard chelating agent supplied by LGC Standards with 98% of purity. This ensures the exclusive production of o,o-EDDHA/Fe³⁺ and it can be used as reference point for the products analysis.

The eluent solution for the HPLC analysis is composed by:

- 350 ml of Acetonitrile;
- 5 ml di TBA⁺ (1 M solution);
- 645 ml of HPLC grade distilled water.

The obtained solution is stabilized at 6 pH, in fact as explained in the literature the pH of mobile phase affects the retention time and the k of calibration method. Because the eluent phase has been prepared more than one times and because it is impossible duplicate exactly the pH, following are presented the calibration curves and the relative constants used during the work.

The first method calibration has been performed dissolving 11.4 mg of standard in 25 ml of sodic solution at concentration of 0.24 g/l of NaOH in distilled water. The standard solution has been chelated with iron (III) nitrate nonahydrate solution at concentration of 89 g/l. During the chelation the pH has been adjusted between 7 and 8, as recommended by the European rule. This solution, indicated S₀, has been diluted as follow:

- S₀ = 11.4 mg EDDHA_{STD} in 25 ml of sodic solution chelated with 89 g/l iron salt solution;
 - S₁ = dilution of S₀ 1:2;
-

- S_2 = dilution of S_0 1:5;
- S_3 = dilution of S_0 1:10;
- S_4 = dilution of S_0 1:25;
- S_5 = dilution of S_0 1:100;

each sample has been analyzed by HPLC and the resulting chromatograms are presented in the picture below:

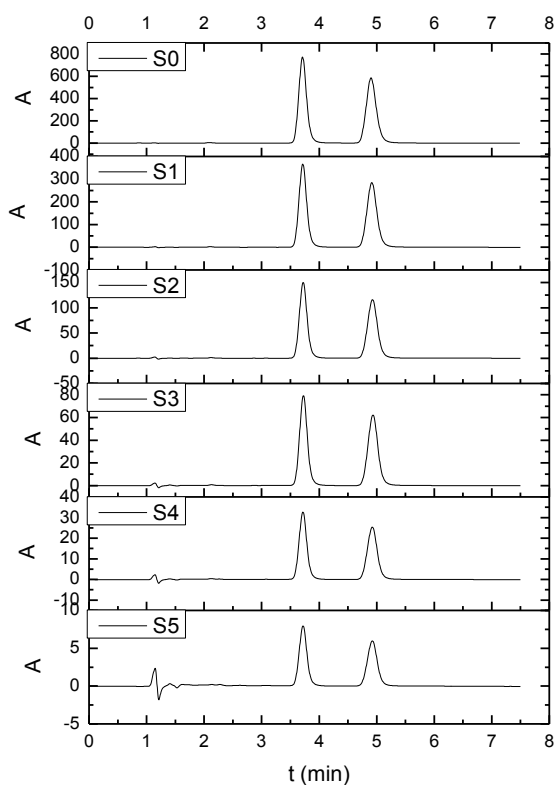


Figure 28. Chromatograms of the dilutions of the first calibration of analytical method

It is possible to note the presence of two peaks which identify respectively, from left to right, racemic and meso isomeric forms.

Relating the concentration of analyzed solution and the area of peaks of chromatograms, it is possible deduce the calibration straight line.

The S_0 concentration has been calculated as follow:

$$C_{S_0} = \frac{m_{S_0} \cdot \frac{PM_{Fe}}{PM_{EDDHA_{STD}}}}{V_{sol}} = \frac{11.4 \text{ mg} \cdot \frac{56 \frac{\text{g}}{\text{mol}}}{360.36 \frac{\text{g}}{\text{mol}}}}{25.8 \text{ ml} \cdot \frac{1 \text{ l}}{1000 \text{ ml}}} = 68.46 \frac{\text{mg}}{\text{l}}$$

where:

- m_{S_0} is the used mass of standard;
- PM_{Fe} is the molecular weight of iron;
- $PM_{EDDHA_{STD}}$ is the molecular weight of standard EDDHA
- V_{sol} is the dissolution volume considering the 25 ml of sodic solution and the acid and base used to reach the 7.5 pH.

The concentration of dilutions can be easily calculate. In the Table 2 are showed the concentrations and the absorbance of each sample analyzed:

Table 2. Absorbance and concentration of the dilutions of the first calibration of analytical method

	C [mg/l]	A ₁	A ₂	A = A ₁ + A ₂
S ₀	68.46	125.87	122.24	248.12
S ₁	34.3	59.46	59.36	118.81
S ₂	13.7	24.35	24.00	48.36
S ₃	6.9	12.84	12.84	25.68
S ₄	2.7	5.30	5.23	10.53
S ₅	0.7	1.28	1.23	2.51

It is possible to show in a graph the trend of absorbance against the concentration for peak 1 and 2:

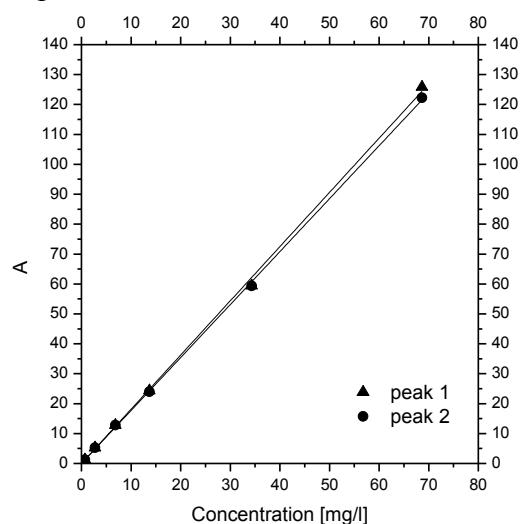


Figure 29. Absorbance vs concentration for peak 1 (triangle) and peak 2 (circle)

By a linear fitting of points, forcing the straight lines to pass through the axis origin, is possible to obtain the following results:

- for the peak 1 $y = 1.812 \cdot x$;
- for the peak 2 $y = 1.770 \cdot x$.

Considering that the ortho-ortho EDDHA is characterized by the contemporary presence of both, meso and racemic, isomers, it is necessary to calibrate the analytical method on the sum of the two peaks:

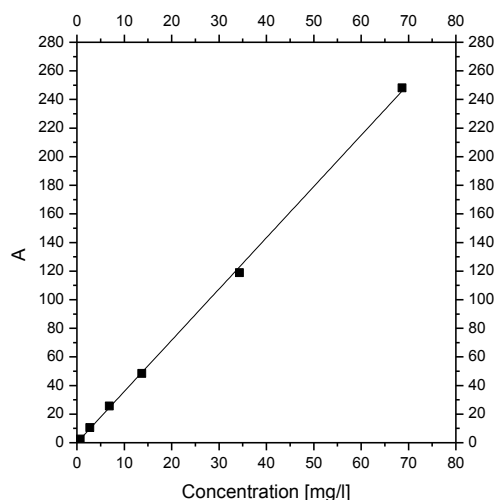


Figure 30. Calibration straight line n° 1

By the linear fitting of points, forcing the straight line to pass through the axes origin, the following equation is obtained: $y = 3.582 \cdot x$, where the slope of straight line represents the calibration constant of the analytical method: $k = 3.582 \frac{l}{mg}$.

The second calibration of analytical method has been obtained by the chelation, with iron (III) nitrate nonahydrate solution at concentration of 89 g/l, of 12.1 mg of standard dissolved in alkali solution at concentration of 0.24 g/l of NaOH in distilled water. In the same way of the first calibration method, the pH has been regulated by the addition of HCl 37% water solution w/w and NaOH 50% water solution w/w in order to reach a value between 7 and 8 pH.

From this solution (S_0), five dilutions have been realized, in the same way of the first calibration method, and the resulting chromatogram are shown in the graph below:

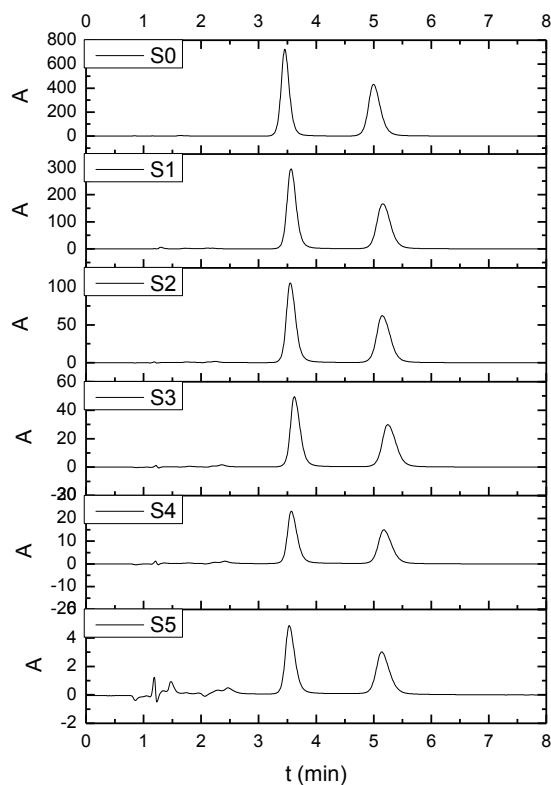


Figure 31. Chromatograms of the dilutions of the second calibration of analytical method

In the next table are showed the concentration of samples and the peaks area:

Table 3. Absorbance and concentration of the dilutions of the second calibration of analytical method

	c [mg/l]	A₁	A₂	A = A₁ + A₂
S₀	67.64	129.53	111.69	241.22
S₁	33.8	59.15	48.17	107.32
S₂	13.5	21.36	17.88	39.23
S₃	6.8	10.25	8.69	18.94
S₄	2.7	4.85	4.21	9.06
S₅	0.7	0.99	0.83	1.83

Also in this case is possible to correlate by a graph the concentration and the peaks area:

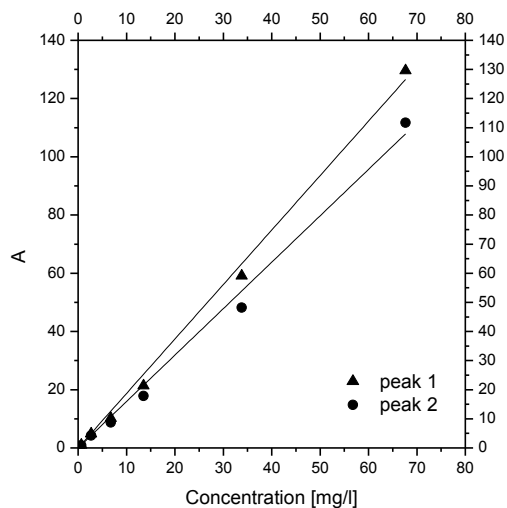


Figure 32. Absorbance vs concentration for peak 1 (triangle) and peak 2 (circle)

By a linear fitting of points, forcing the straight lines to pass through the axis origin, is possible to obtain the following results:

- for the peak 1 $y = 1.871 \cdot x$;
- for the peak 2 $y = 1.595 \cdot x$;

where the slope of regression equation is the calibration constant for each isomeric form. Correlating the sum of peaks area and the solution concentrations is possible to calculate the calibration constant for both isomeric form and this can be used as k constant in the Lambert Beer law in the analysis of synthesized o,o -EDDHA/ Fe^{3+} :

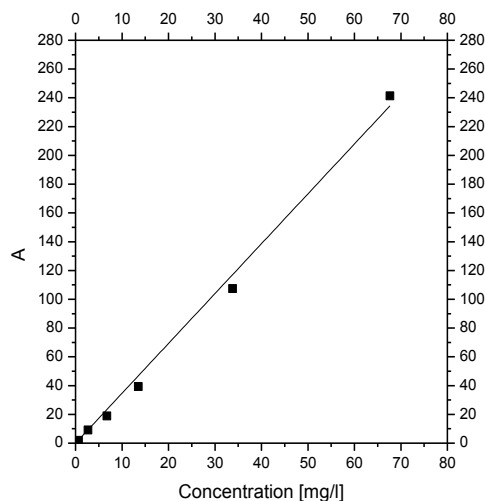


Figure 33. Calibration straight line n° 2

The equation of the second calibration straight line is: $y = 3.465 \cdot x$, with a calibration constant $k = 3.465 \frac{l}{mg}$

Other three times has been necessary the calibration of the analytical method and in particular, two times as iron salt for the chelation has been used the iron (III) nitrate nonahydrate and in the last one has been used the iron (III) sulfate.

The third calibration of the analytical method has been performed dissolving

The third method calibration has been performed dissolving 13.3 mg of standard in 25 ml of sodic solution at concentration of 0.24 g/l of NaOH in distilled water. The standard solution has been chelated with iron (III) nitrate nonahydrate solution at concentration of 89 g/l. During the chelation the pH has been adjusted between 7 and 8, as recommended by the European rule. From this solution, indicated as S_0 , has been obtained five dilutions as described before.

The chromatograms obtained by the HPLC analysis are showed in the picture below:

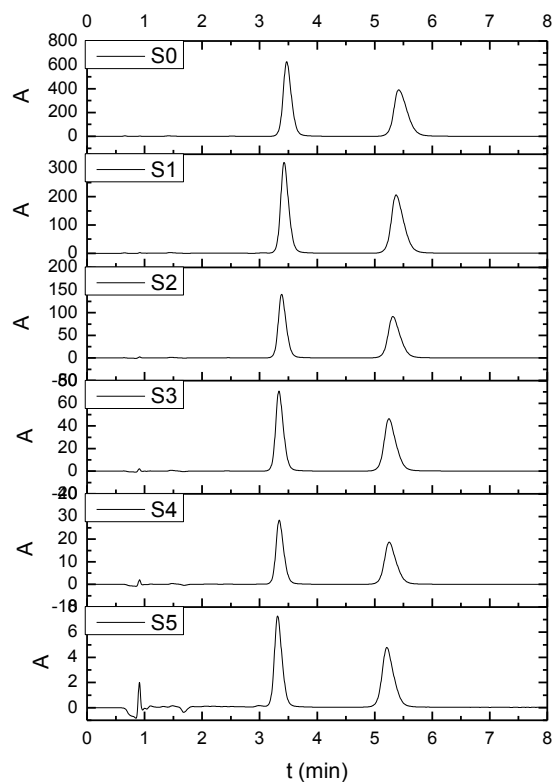


Figure 34. Chromatograms of the dilutions of the third calibration of analytical method

Considering the 0.5 ml of base used during the chelation to reach the 7.5 value, the S_0 concentration is:

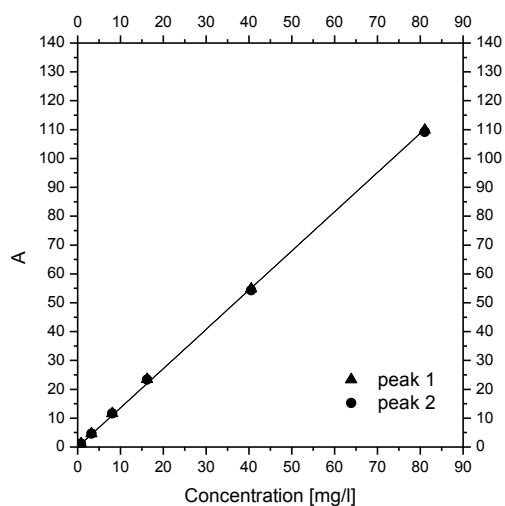
$$C_{S_0} = \frac{m_{S_0} \cdot \frac{PM_{Fe}}{PM_{EDDHA_{STD}}}}{V_{sol}} = \frac{13.3 \text{ mg} \cdot \frac{56 \frac{g}{mol}}{360.36 \frac{g}{mol}}}{25.5 \text{ ml} \cdot \frac{1 \text{ l}}{1000 \text{ ml}}} = 81.05 \frac{\text{mg}}{\text{l}}$$

By the data obtained with the HPLC analysis, the concentration of each sample has been related with the absorbance:

Table 4. Absorbance and concentration of the dilutions of the third calibration of analytical method

	C [mg/l]	A ₁	A ₂	A = A ₁ + A ₂
S ₀	81.05	109.90	109.20	219.09
S ₁	40.5	54.86	54.32	109.18
S ₂	16.2	23.53	23.37	46.90
S ₃	8.1	11.63	11.64	23.27
S ₄	3.2	4.65	4.67	9.32
S ₅	0.8	1.18	1.18	2.36

It is possible to show in a graph the trend of absorbance against the concentration for peak 1 and 2:

**Figure 35. Absorbance vs concentration for peak 1 (triangle) and peak 2 (circle)**

By a linear fitting of points, forcing the straight lines to pass through the axis origin, is possible to obtain the following results:

- for the peak 1 $y = 1.359 \cdot x$;
- for the peak 2 $y = 1.350 \cdot x$;

from which are obtained the calibration constant for the evaluation of both isomers separated. By the correlation between dilutions concentration and the sum of peaks area is possible to calculate the

calibration constant can be used as k constant in the Lambert Beer law in the analysis of synthesized o,o-EDDHA/Fe³⁺:

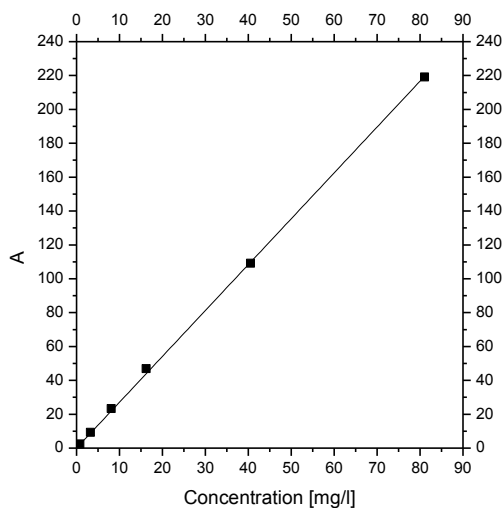


Figure 36. Calibration straight line n° 3

The equation of the third calibration straight line is: $y = 2.709 \cdot x$ and the $k=2.709 \frac{l}{mg}$.

The 4th calibration of the method has been performed dissolving 8.3 mg of standard in 25 ml of sodic solution at concentration of 0.24 g/l of NaOH in distilled water. The standard solution has been chelated with iron (III) nitrate nonahydrate solution at concentration of 89 g/l. During the chelation, the pH has been adjusted between 7 and 8 using 2.7 ml of acid and base. From this S₀ standard solution has been produced five dilutions, according the dilution ratios described previously, and the relative chromatograms are showed below:

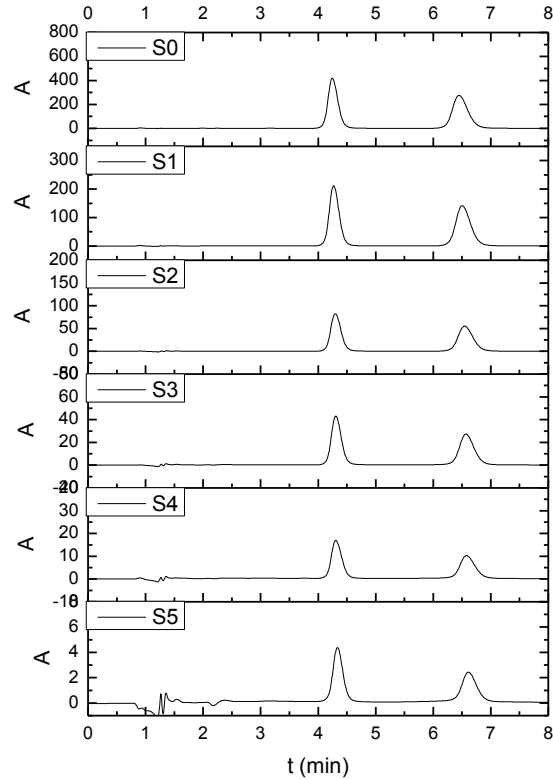


Figure 37. Chromatograms of the dilutions of the 4th calibration of analytical method

The S_0 concentration is:

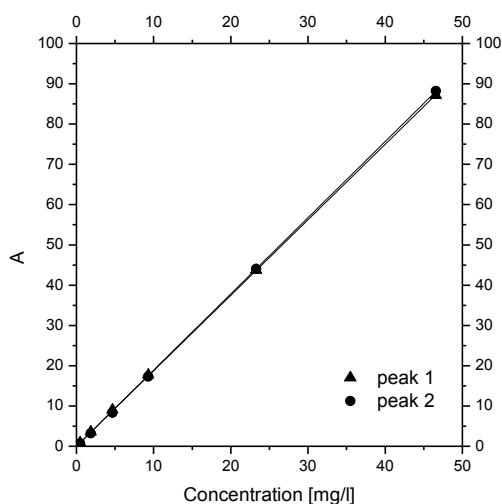
$$C_{S_0} = \frac{8.7 \text{ mg} \cdot \frac{56 \frac{\text{g}}{\text{mol}}}{360.36 \frac{\text{g}}{\text{mol}}}}{27.7 \text{ ml} \cdot \frac{1 \text{ l}}{1000 \text{ ml}}} = 46.56 \frac{\text{mg}}{\text{l}}$$

By the data obtained with the HPLC analysis, the concentration of each sample has been correlated with the absorbance:

Table 5. Absorbance and concentration of the dilutions of 4th calibration of analytical method

	C [mg/l]	A ₁	A ₂	A = A ₁ + A ₂
S ₀	46.56	87.13	88.23	175.36
S ₁	23.3	43.74	44.01	87.75
S ₂	9.3	17.77	17.32	35.09
S ₃	4.7	9.01	8.34	17.35
S ₄	1.9	3.58	3.17	6.75
S ₅	0.5	0.89	0.75	1.64

It is possible to show in a graph the trend of absorbance against the concentration for peak 1 and 2:

**Figure 38. Absorbance vs concentration for peak 1 (triangle) and peak 2 (circle)**

By a linear fitting of points, forcing the straight lines to pass through the axis origin, is possible to obtain the following results:

- for the peak 1 $y = 1.874 \cdot x$;
- for the peak 2 $y = 1.892 \cdot x$;

from which are obtained the calibration constant for the evaluation of both isomers separated. By the correlation between dilutions concentrations and the sum of peaks area is possible to calculate the

calibration constant can be used as k constant in the Lambert Beer law in the analysis of synthesized o,o -EDDHA/ Fe^{3+} :

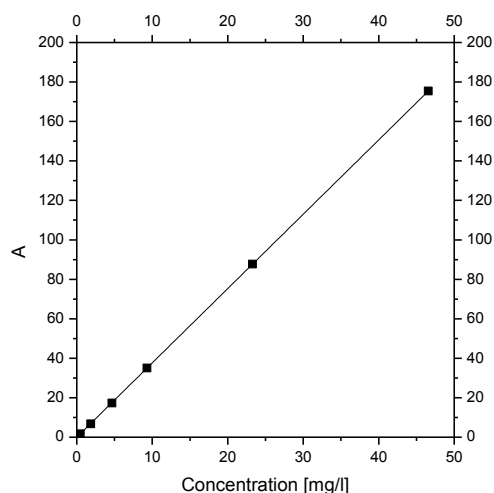


Figure 39. Calibration straight line n° 4

The equation of the third calibration straight line is: $y = 3.766 \cdot x$ and the $k=3.766 \frac{l}{mg}$.

The last calibration of the analytical method has been performed dissolving 20.1 mg of standard in 25 ml of sodic solution at concentration of 0.24 g/l of NaOH in distilled water. The standard solution has been chelated with iron (III) sulfate solution at concentration of 89 g/l. During the chelation, the pH has been adjusted between 7 and 8 using 1.8 ml of acid and base. From this S_0 standard solution has been produced six dilutions:

- S_1 = dilution of S_0 1:2;
- S_2 = dilution of S_0 1:5;
- S_3 = dilution of S_0 1:10;
- S_4 = dilution of S_0 1:25;
- S_5 = dilution of S_0 1:50;
- S_6 = dilution of S_0 1:100.

The chromatograms obtained are showed in the picture below:

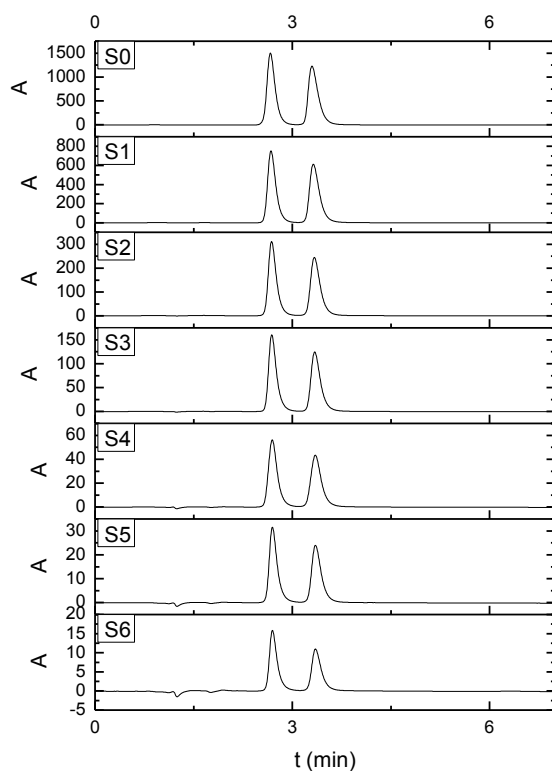


Figure 40. Chromatograms of the dilutions of the 5th calibration of analytical method

As it possible to note, the time analysis has been reduce considering that after 7 minutes both the peaks relative to the isomeric form of EDDHA are present in the chromatograms.

The S_0 concentration is:

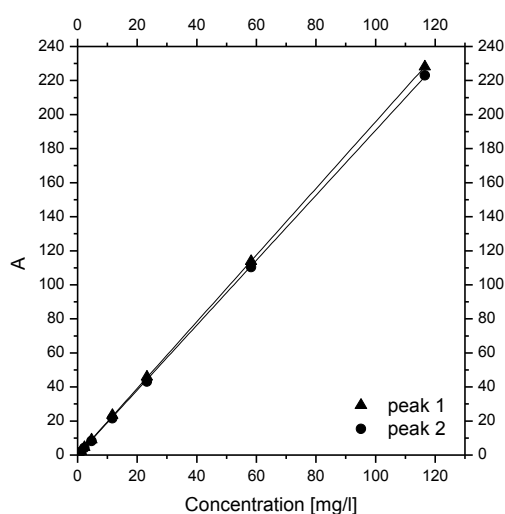
$$c_{S_0} = \frac{20.1 \text{ mg} \cdot \frac{56 \frac{\text{g}}{\text{mol}}}{360.36 \frac{\text{g}}{\text{mol}}}}{26.8 \text{ ml} \cdot \frac{1 \text{ l}}{1000 \text{ ml}}} = 116.65 \frac{\text{mg}}{\text{l}}$$

By the data obtained with the HPLC analysis, the concentration of each sample has been correlated with the absorbance:

Table 6. Absorbance and concentration of the dilutions of the 5th calibration of analytical method

	C [mg/l]	A ₁	A ₂	A = A ₁ + A ₂
S ₀	116.55	228.13	223.02	451.15
S ₁	58.3	113.93	110.41	224.34
S ₂	23.3	46.00	43.13	89.13
S ₃	11.7	23.39	21.61	45.00
S ₄	4.7	9.08	8.17	17.25
S ₅	2.3	4.62	4.18	8.79
S ₆	1.2	2.27	1.89	4.16

It is possible to show in a graph the trend of absorbance against the concentration for peak 1 and 2:

**Figure 41. Absorbance vs concentration for peak 1 (triangle) and peak 2 (circle)**

By a linear fitting of points, forcing the straight lines to pass through the axis origin, is possible to obtain the following results:

- for the peak 1 $y = 1.958 \cdot x$;
- for the peak 2 $y = 1.907 \cdot x$;

from which are obtained the calibration constant for the evaluation of both isomers separated. By the correlation between dilutions

concentration and sum of peaks area is possible to calculate the calibration constant can be used:

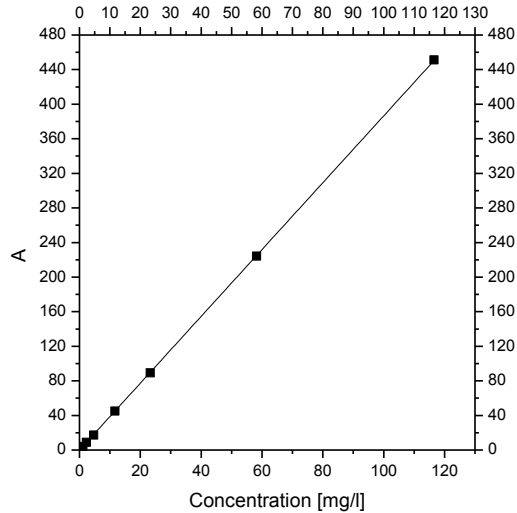


Figure 42. Calibration straight line n° 5

The equation of the 5th calibration straight line is: $y = 3.865 \cdot x$ and the $k=3.865 \frac{l}{mg}$

The aim of the above described work is obtain the k of Lambert Beer law in order to calculate the concentration of analyzed samples when the concentration is unknown. In this case is possible to calculate the iron ortho ortho chelated concentration as:

$$C_{Fe} = \frac{A_1}{k_1} + \frac{A_2}{k_2} = \frac{A}{k} \left[\frac{mg}{l} \right]$$

2.4.2 Products analysis and material balance

By the elaboration of obtained data from the analysis is possible calculate the reaction yield.

Following the definition of used entities:

- m_p [g], weight of powders obtained with drying;
 - m_A [g], mass of analyzed powder (about 60 mg);
 - V [ml], alkali solution volume used to dissolve the m_A (50 ml);
 - $V_{reagents}$ [ml], reagents volume in the reactor;
 - V_{sample} [ml], sample volume took during reaction;
-

- $MM_{Fe} = 56$ [g/mol], iron molecular weight;
- n_{rl} , number of moles of limiting reagents.

Known the above parameters is possible to calculate:

- analyzed powder concentration:

$$C_P = \frac{m_A}{V} \left[\frac{mg}{l} \right]$$

- mole of chelated iron in the sample:

$$n_{Fe}^{sample} = C_{Fe} * \frac{V}{MM_{Fe}} \text{ [mol]}$$

- EDDHA chelated with iron percentage:

$$\%_{EDDHA_{chelated}} = \frac{C_{Fe}}{C_P} * 100$$

- iron moles in the sample:

$$n_{Fe}^{sample\ taking} = \frac{n_{Fe}^{sample} * m_P}{m_A}$$

- iron moles in the reactor:

$$n_{Fe}^{reactor} = \frac{n_{Fe}^{sample\ taking} * V_{reagents}}{V_{sample}}$$

Considering the reaction product, $n_{Fe}^{sample\ taking} = n_{Fe}^{reactor}$ because $V_{reagents} = V_{sample}$.

- EDDHA reaction yield:

$$\eta = \frac{n_{Fe}^{reactor}}{n_{rl}} * 100$$

Moreover for each synthesis, the material balances have been verified to validate the correct working of system. Considering the schematization of plant as follow:

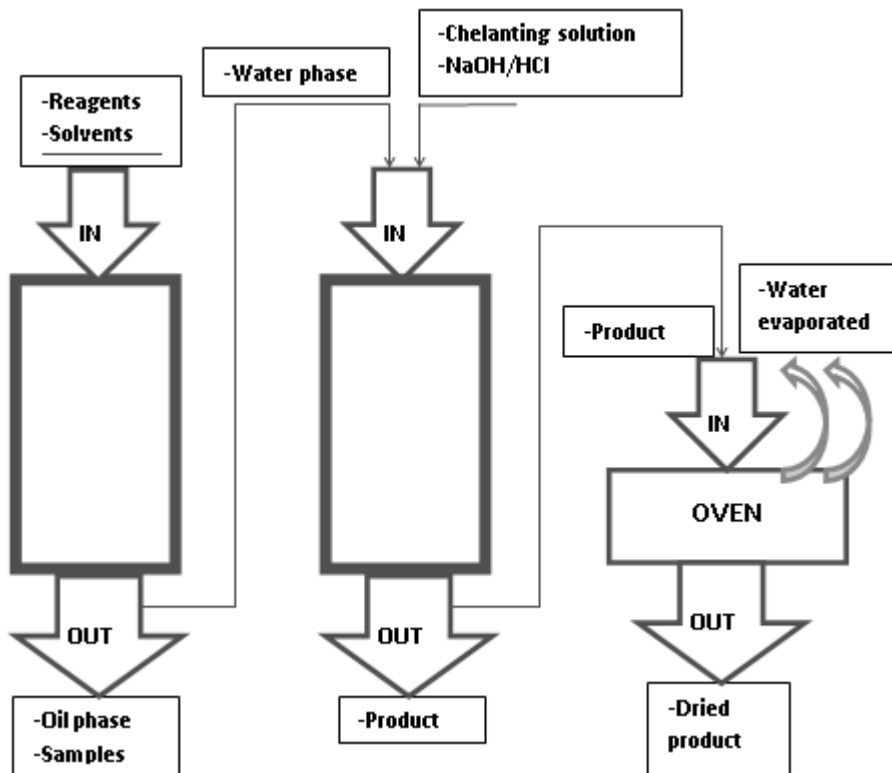


Figure 43. Plant scheme

And considering the control volume as the complete plant, the material balance is:

$$\text{IN}-\text{OUT}+\text{GEN}=\text{ACC}$$

2.6 Image analysis

In order to evaluate the efficacy of the treatment with the iron chelates, a test on a seedling of lettuce plants have been performed. The experimental phase, of the duration of 43 days, has been carried out using three different batches of lettuce plants, starting from the same chlorotic conditions and treated as follow:

- untreated, the plants have been only watered every day for 43 days;
- commercial, the plants have been watered every day for 43 days and every 5 day they have been treated with commercial iron chelate;

- T5A, the plants have been watered every day for 43 days and every 5 day they have been treated with iron chelate synthesized in laboratory.

The samples have been photographed every day to check the possible progress of iron chlorosis therapy and to evaluate the progressive yellowing and the death of plants.

Chapter Four

Results and discussion: process analysis

Part of this work has been reported in:

Apicella P., Cascone, S., Lamberti G., De Santis F., “Iron chelates: production processes and reaction evolution analysis”, Chemical Engineering Communications, 2015, DOI: 10.1080/00986445.2015.1114476.

4.1 EDDHA production process: evaluation of process conditions

The syntheses performed have been focused on the production of o,o-EDDHA/Fe³⁺, starting to the reaction process described in the chapter three and applying some modifications in order to understand completely the process and the parameters by which the process can be modified and optimized according our aims.

4.1.1 Syntheses #1

According to the production process described in the paragraph 2.4.1, to the synthesis reactor has been fed the following reagents quantities:

Table 7. Reagents fed for the synthesis #1

	Mole	Weight [g]	Volume [ml]
Phenol	3.9	367.03	343.03
Ethylenediamine	0.15	9.02	10.02
NaOH	0.3	12	16*
Glyoxylic acid	0.3	22.21	33.15*
Reagents Volume (V_{reagents})			403.19

* 50% w/w solution

At the end of reaction phase, the extraction phases has been performed by three washing, the first one with both toluene and water and the other ones only with the organic solvent.

Table 8. Solvents used to separate the water and oil phases

Washing		Volume [ml]
1° washing	Water	500
	Toluene	900
2° washing	Toluene	700
3° washing	Toluene	500

The extraction of phases of samples taken during the reaction has been realized using 2 ml of water and 5 ml toluene for each sample. The water phase obtained by the separation of phases has been chelated

with an iron (III) nitrate nonahydrate concentrated solution prepared according the quantities of Table 9.

Table 9. Iron (III) nitrate nonahydrate solution composition

	Weight [g]
Water	200
Iron (III) nitrate nonahydrate	87.18

At the end of chelation the product has been collected and dried in oven.

The production of o,o-EDDHA/Fe³⁺ according the reference production process has been performed in triplicate, in order to validate the results and each batch has yielded the following amount of powders:

- batch#1 96.64 g of powder;
- batch#2 128.14 g of powder;
- batch#3 86.94 g of powder.

Products chromatographic analysis

After the drying, about 60 mg of product (m_A) has been dissolved in 50 ml (V) of alkali solution at concentration of 0.24 g/l of NaOH in water. In the same way, the samples extracted every 10 minutes has been dissolved in mild caustic solution and analyzed by HPLC, according the methods described in the chapter three.

The analytical determinations, on the products obtained from the three batches of syntheses #1, have confirmed the reproducibility of the process results. The typical chromatogram of product of syntheses #1 is showed in the graph below:

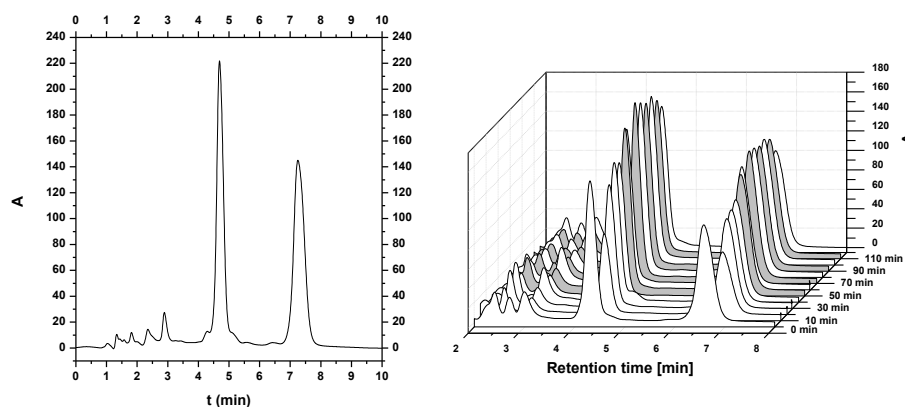


Figure 44. Chromatogram of the product of the syntheses #1 (on the left) and absorbance vs retention time with the proceed of the syntheses #1 reaction time (on the right)

From the chromatogram is clear the presence of both the isomeric form of o,o -EDDHA/ Fe^{3+} and in particular, the rac - o,o -EDDHA/ Fe^{3+} is represented by the first peak between 4 and 5.5 minutes, while the second peak, between 6.5 and 8.5 minutes, highlights the presence of the $meso$ - o,o -EDDHA/ Fe^{3+} .

As it is possible to note, the chromatogram of product is very clean and only few amounts of unreacted reagents and undesired products of small dimension, proved by the small peaks at the beginning of the chromatographic analysis, are present.

Unfortunately it is not possible compare this result with Petree et al. patent [29] result because in the patent is not present any form of graph but only the numeric result of analysis.

From the analysis of samples taken during the reaction is possible achieve some useful kinetics information, in fact plotting the retention time on the abscissa, the signal intensity on the ordinate and the reaction time on the z axis, is possible to have an idea of the evolution of reaction products with the reaction time. The area calculation allowed the exact quantification of the o,o -EDDHA/ Fe^{3+} content. From the chromatograms evolution is possible to quantify the evolution of the reaction by the increasing of the peaks areas, relative to the rac emic and $meso$ o,o -EDDHA/ Fe^{3+} . Comparing the areas of the peaks, the reaction of pattern is immediately visible: at the beginning of the reaction the product in the reacting mixture significantly increases with the time; later, the difference between the

areas of the peaks are negligible, indicating that the reaction probably is ended. Because by the peaks area is possible to calculate the reaction yield and the ortho-ortho chelated iron fraction, as reported in chapter three, the yield evolution with time permits to find out the desired information about the reaction development.

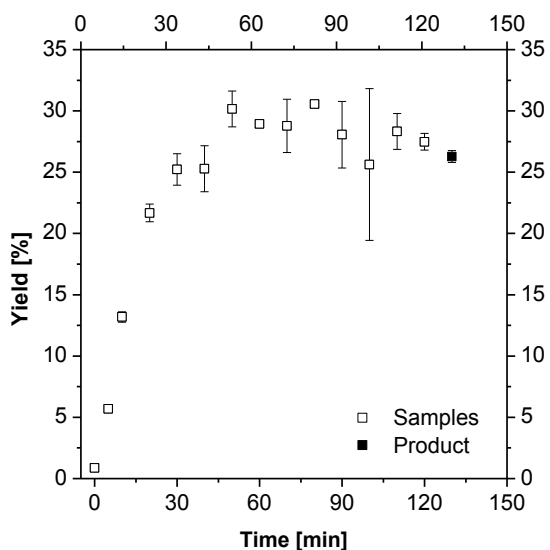


Figure 45. Yield evolution with reaction time for the syntheses #1

In the Figure 45 the reaction yield evolution is shown, where the samples from 0 to 120 minutes represent the results of the analysis of the samples withdrawn from the reactor, whereas the last point (the empty square) represents the reaction yield obtained from the analytical determination on the product. The synthesis has been reproduced in triplicate and the results are shown in terms of mean value between the samples, the vertical error bars indicate the standard deviation between the samples of each synthesis.

It has to be noted that the separation and the chelation process for each sample have been conducted separately, thus is very difficult to reproduce exactly the same conditions, this leads to an high variability between the samples and between the samples and the product. By the Figure 45 is clear that the reaction evolves rapidly in the product formation during the first 30 minutes, then its kinetics proceeds slowly and, after about 50 minutes of the reaction a plateau value is reached. This evolution confirms that the reaction time is much lower than that indicated in literature, which means that, according with this analysis,

a lot of time can be saved, thus increasing the productivity of an eventual industrial processes. At the end of the process, an average yield of 27% and an o,o-EDDHA/Fe³⁺ amount of 2.6% have been obtained.

4.1.2 Syntheses #2

In order to pursue the aim to reduce the phenol excess in a global reduction of solvent use, the syntheses #2 has been realized modifying the feed ratio proposed by Petree [29] and in particular, the phenol excess has been drastically reduced and it has been partially substituted with toluene which it acts as solvent only. Therefore the molar ratio of the syntheses #2 are:

Phenol: Toluene: Ethyl. : NaOH: Glyoxylic acid = 3.6: 1.2: 0.5: 1: 1

The volumes of reagents fed have been increased, respect to the syntheses #1, with the aim to increase the obtained product.

Another difference with Petree patent [29] is that in syntheses #2 only one washing during the separation phase has been performed.

In the same way than synthesis #1, the reagents has been fed to the reactor and after this phase (30 minutes at 45°C internal reactor temperature) the inner reactor temperature has been increased to 75°C for the two hours of reaction. To perform the kinetics analysis, every 10 minutes 2 ml sample has been taken from the reactor.

In the following table are showed the reagents quantities used:

Table 10. Reagents fed for the syntheses #2

	Mole	Weight [g]	Volume [ml]
Phenol	3.63	341.91	322.56
Toluene	1.21	111.65	128.33
Ethylenediamine	0.48	29	32.26
NaOH	0.97	77.72	51.81*
Glyoxylic acid	0.97	143.26	106.75*
Reagents Volume (V_{reagents})			641.71

* 50% w/w water solution

The extraction phases step has been modified according the goal to save the use of organic solvent and in fact one only wash with water and toluene has been performed and the toluene volume used has been further reduced, considering the toluene fed to the reaction:

Table 11. Solvents fed for the extraction phases

	Volume [ml]
Water	1160
Toluene	128.33

The separation of phases for the sample taken during the reaction has been performed using a solution of 3.6 ml of water and 0.4 ml of toluene for each sample.

After the extraction of water and oil phases from the synthesis reactor the water phase has been subjected to the chelation with a concentrated iron (III) nitrate nonahydrate solution.

Table 12. Quantity used to prepare chelating solution

	Weight [g]
Water	145
Iron (III) nitrate nonahydrate	126.15

After the addition of iron concentrated solution the pH has been rapidly stabilized to the set point value of 7.5 in fact after only 4 minutes the mixture pH was 7.5.

In the same way the water phase extracted from the sample taken every 10 minute has been chelated with 3 ml of chelating solution at the same concentration of solution used for the chelation of water phase in the chelation reactor.

The syntheses #2 has been reproduced three times to guarantee the repeatability of data and from each batch has been obtained the following amounts of powder:

- batch #1 268.66 g of powder;
 - batch #2 279.58 g of powder;
 - batch #3 257.46 g of powder.
-

Products chromatographic analysis

After drying about 60 mg of product (m_A) has been dissolved in 50 ml (V) of alkali solution at concentration of 0.24 g/l of NaOH in water. In the same way, the sample extracted every 10 minutes has been dissolved in mild caustic solution and analyzed by HPLC.

From the analysis of products obtained applying the protocol of the syntheses #2, it has been the following chromatogram:

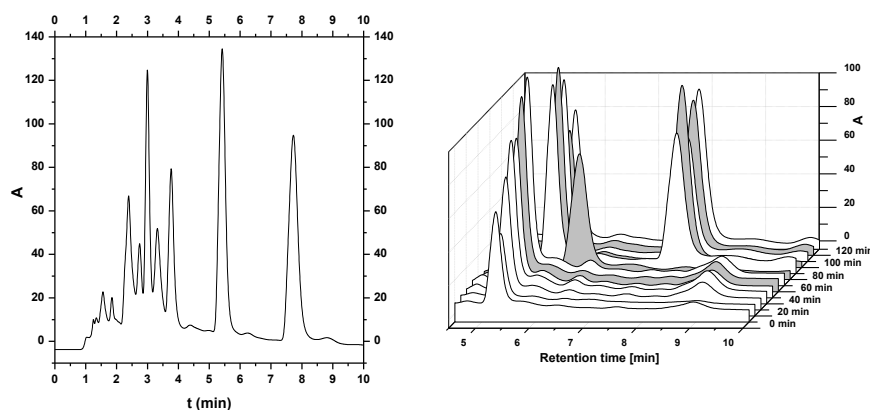


Figure 46. Chromatogram of product of the synthesis #2 (on the left) and absorbance vs retention time with the proceed of the synthesis #2 reaction time (on the right)

From the chromatogram is clear the presence of the two diastereoisomeric form of o,o-EDDHA/ Fe^{3+} racemic and meso, respectively at 5.5 and 8 minutes, but at the same time the other peaks show clearly the presence of o,p-EDDHA/ Fe^{3+} (3.7 minutes) and the other polycondensation products that cannot be better characterized.

From the analysis of the sample taken during the reaction can be obtained some interesting kinetics information, in fact also in this case, observing the 3D plot in Figure 45 is possible to have an idea of the evolution of products formation with the reaction time. By the comparison of the areas of the peaks, it is clear as at the beginning of the reaction the product in the reacting mixture significantly increases with the time and this growth characterized the first 60 minutes of reaction; later, the difference between the areas of the peaks decrease, indicating that the reaction probably is ended. Because by the peaks area is possible to calculate the reaction yield and the ortho-ortho chelated iron fraction, the yield evolution with time permits to find out the desired information about the reaction development.

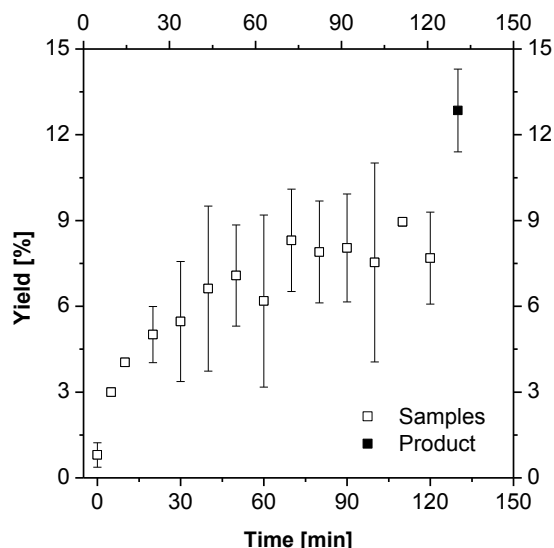


Figure 47. Yield evolution with reaction time for the syntheses #2

In the Figure 47 is shown the reaction yield evolutions, where the samples from 0 to 120 minutes represent the results of the analysis of the samples taken from the reactor, whereas the last point (the empty square) represents the reaction yield obtained from the analytical determination on the product. The synthesis has been reproduced in triplicate and the results are shown in terms of mean value between the samples, the vertical error bars indicate the standard deviation between the samples of each synthesis.

The presence of the error bars is caused by the before explained difficulties to reproduce exactly the same conditions for the withdrawn samples.

Unfortunately the high value of the error bars does not permit an exact evaluation of reaction kinetic, nevertheless by the Figure 47 is possible to note a trend of the reaction yield which evolves rapidly in the product formation during the first 30-40 minutes, then its kinetics proceeds slowly and, after about 60 minutes of the reaction a plateau value is reached.

Also this analysis of reaction evolution confirms that the reaction time is much lower than that indicated in literature, and this corroborate the idea that the reaction time can be decreased, a lot of time can be saved, and an increasing of the productivity, of an eventual industrial process, can be realized.

The syntheses #2 results in an average yield of 12% and an o,o-EDDHA/Fe³⁺ fraction of 1.2%.

4.1.3 Syntheses #3

According to the literature, the syntheses #3 has been performed testing the effect of a catalyst and in particular the manganese (II) acetate tetrahydrate, one of the suggested catalysts by Jullien patent [30], has been used.

The syntheses #3 have followed the reaction condition described for the syntheses #1, feeding the reagents with the same molar ratio while the catalyst has been fed, following the suggestion of Jullien patent [30], with a molar ratio in the range 0.001-0.05 mole of catalyst for each mole of glyoxylic acid. The catalyst has been dosed 10 minutes after the phenol.

Also for the syntheses #3 every 10 minutes has been performed a withdrawing for the kinetics analysis.

Table 13. Reagents fed for the syntheses #3

	Mole	Weight [g]	Volume [ml]
Phenol	3.9	367.029	343.018
Ethylenediamine	0.15	9.015	10.0167
NaOH	0.3	12	16*
Glyoxylic acid	0.3	22.212	33.152*
Catalyst	0.006	1.6	
Reagents Volume (V_{reagents})			641.71

* 50% w/w water solution

At the end of reaction the reactor has been cooled down and the phases extraction has been performed. Based on the experience of previous syntheses #1, only two extractions with water and toluene have been realized because it has been observed that the oil phase obtained from the third washing is exactly the toluene fed to promote the separation, with no considerable change in color and physical characteristics.

Moreover, still with the global goal to reduce the solvent use and post processing operations, the volumes of solvents used to promote the phases separation have been reduced.

Table 14. Solvents fed for the separation phases

Washing		Volume [ml]
1° washing	Water	500
	Toluene	750
2° washing	Water	100
	Toluene	300

The phases separation for the sample taken during the reaction has been performed using a solution of 3 ml of water and 6 ml of toluene for each sample, according to the proportion adopted for the production process.

The syntheses #3 has been reproduced three times to validate the results, but only the chelation of water phase of the first batch has been performed with a solution at the same iron concentration of syntheses #1 (40% of salt excess respect the 100% of reaction yield), whereas the second and the third batches have been chelated with an iron solution obtaining considering a salt excess of 20% respect the 60% of theoretical yield of Petree et al. patent [29], in order to evaluate the effects of iron salt excess on the syntheses results.

Table 15. Quantity used to prepare chelating solution of the first batch (on the top) and of the second and the third batch (on the bottom)

	Weight [g]
Water	145
Iron (III) nitrate nonahydrate	87.17

	Weight [g]
Water	145
Iron (III) nitrate nonahydrate	44

After the addition of iron concentrated solution the pH rapidly goes down at 3.5. In order to fast reach the pH 7.5, 6 ml of caustic soda 50% w/w water solution has been manually introduced in the chelation reactor.

In the same way the water phase extracted from the sample taken every ten minute has been chelated with 3 ml of chelating solution at

the same concentration of solution used for the chelation of water phase in the chelation reactor.

After the drying, from each batch has been recovered the following powders amounts:

- batch#1 116.03 g of powder;
- batch#2 79.15 g of powder;
- batch#3 77.1 g of powder.

It is important to highlight as the batch#2 and #3 have been realized with a less iron excess respect to the batch#1.

Products chromatographic analysis

After drying about 60 mg of product (m_A) has been dissolved in 50 ml (V) of alkali solution at concentration of 0.24 g/l of NaOH in water. In the same way, the sample extracted every 10 minutes has been dissolved in mild caustic solution and analyzed by HPLC.

From the analysis of products of syntheses #3, it has been obtained the following characteristic chromatogram:

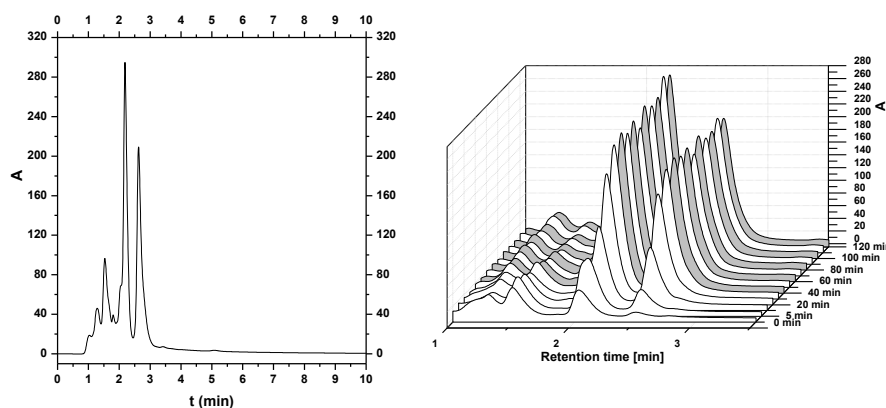


Figure 48. Chromatogram of product of the syntheses #3 (on the left) and absorbance vs retention time with the proceed of the syntheses #3 reaction time (on the right)

The chromatogram of product of the syntheses #3 highlights the presence of both the diastereoisomeric form of o,o -EDDHA/ Fe^{3+} which have been detected after 2.1 minutes, the racemic- o,o -EDDHA/ Fe^{3+} , and 2.6 minutes, the $meso$ - o,o -EDDHA/ Fe^{3+} . The

chromatogram is rather clean and only few amount of impurities have been detected at the beginning of the analysis.

From the 3D graph of Figure 48 (on the right) is possible to have an idea of the evolution of reaction with the reaction time while, by the area calculation is allowed the exact quantification of the o,o-EDDHA/Fe³⁺ content. From the chromatograms evolution and comparing the area of peaks is immediately clear the reaction pattern: at the beginning of the reaction the product in the reacting mixture drastically increases with the time; later, after about the first hour of reaction, the difference between the areas of the peaks are negligible, indicating that the reaction evidently is ended. Considering that by the peaks area is possible to calculate the reaction yield and the ortho-ortho chelated iron fraction, the yield evolution with time permits to find out the desired information about the reaction development:

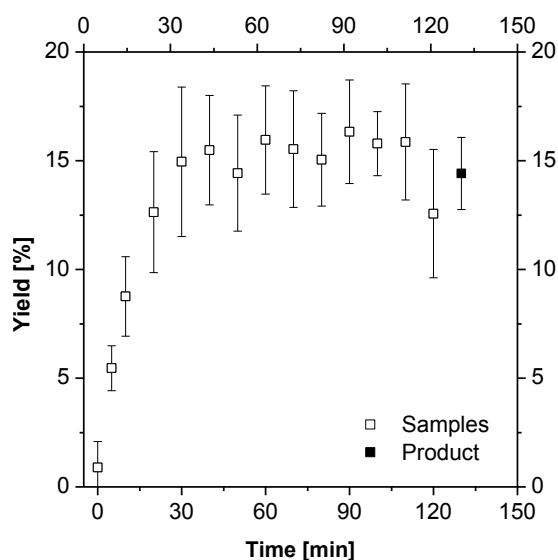


Figure 49. Yield evolution with reaction time for the syntheses #3

Nevertheless, By the reaction yield evolution of Figure 49 is clear that the reaction evolves rapidly in the product formation during the first 30 minutes, then its kinetics proceeds slowly and, after 50 minutes of the reaction a plateau value is reached. This evolution confirms, as already confirmed by the analysis of the results of synthesis #1 and #2, that the reaction time is much lower than that indicated in literature, which means that, according with this analysis, a lot of time can be saved, thus increasing the productivity of eventual industrial

processes. At the end of the process, an average yield of 15% and an o,o-EDDHA/Fe³⁺ amount of 1.5% have been obtained.

**Results and discussion:
reaction optimization**

Part of this work has been reported in:

P. Apicella, S. Cascone, D. Caccavo, G. Lamberti, A. A. Barba, “Optimization of chelates production process for agricultural administration of inorganic micronutrients”, *Chemical Engineering Transaction*, 44 217-222 (2015), doi: 10.3303/CET1544037.

The results of the first part of experimental work have been fundamental to the best understanding of production process of EDDHA chelated iron, of the key parameters and of the critical parts of process.

In fact, from the first three reaction schemes tested, and presented in the chapter 4, it has been possible to understand:

- the kinetic evolution of EDDHA synthesis reaction, such as to set the necessary time to reaction end;
- the necessity to study the best molar ratio between organic solvents and limiting reactant fed, with the partial substitution of phenol with toluene;
- the toluene, as organic solvent to promote the phases separation, is suitable and only two steps of extraction, with solvents volume less than reference production process, are sufficient to guarantee a correct phases splitting;
- the iron (III) nitrate nonahydrate is an adequate iron salt for the chelation and acting on the iron salt excess is possible to regulate the powder quantity obtained and the fraction of ortho-ortho iron chelated.

Based on these information and with the aim to develop an optimized process, a deep screening of all production process phases has been necessary.

5.1 Feeding ratio

The starting point of the process phases screening has been the reagents feeding and in particular the molar ratio organic solvent/ethylenediamine. Four different feeding conditions have been tested with the aim to find the condition which guarantees the best result, in terms of yield and ortho-ortho fraction, using the less quantity as possible of organic solvent.

5.1.1 Synthesis #4

The synthesis #4 has been performed considering the best, from the aim of work point of view, feeding condition: the phenol has been fed stoichiometrically and organic solvent excess has been guaranteed by the toluene, so that the molar ratios of reagents fed have been:

Phenol: Toluene: Ethyl.: NaOH: Glyoxylic acid = 0.3: 3.6: 0.15: 0.3: 0.3

The reagents amount fed is showed in the following table:

Table 16. Reagents fed for the synthesis #4

	Mole	Weight [g]	Volume [ml]
Phenol	0.3	28.233	26.385
Toluene	3.6	331.704	383.472
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			469.028

* 50% w/w water solution

The reaction has been carried out at $75\pm 5^{\circ}\text{C}$ for 120 minutes and at the end of reaction time the reactor has been cooled down and the extraction of phases has been performed. The toluene volume used has been reduced and in particular, the volume of toluene used for the first washing in the syntheses #3 has been reduced considering that a part of toluene has been fed with the reagents and, it does not participate to the reaction, since it acts as solvent only, it can explicate the function of organic solvent to promote the separation of water and oil phase present in the reactor.

The volumes used for the extraction of phases are presented in the following table:

Table 17. Solvents volumes used for the extraction phase of synthesis #4

Washing		Volume [ml]
1° washing	Water	500
	Toluene	350
2° washing	Water	100
	Toluene	300

With the global goal to save the use of hazardous substances, as organic solvent of the 2nd washing has been used a recycled toluene of the second washing of the others syntheses. This operation has been possible considering the low contamination of organic solvent extracted from the second washing.

Also for the synthesis #4 has been performed the sampling of 2 ml every 10 minutes (a sample after 5 minutes of reaction has been taken to best understand the first stage of reaction) and for the extraction of phases has been used 2.5 ml of water and 2.8 ml of toluene.

The water phase has been transferred in the second reactor and has been chelated with an iron (III) nitrate nonahydrate solution with an excess of 20% respect the theoretical reaction yield of 60%:

Table 18. Chelating solution composition of synthesis #4

	Weight [g]
Water	200
Iron (III) nitrate nonahydrate	43.62

During the chelation, 9 ml of sodium hydroxide 50% w/w water solution have been added to rapidly increase the pH in the range of set point value and in fact in 4 minutes and 10 seconds the pH has been stabilized at 7.3 value.

About the samples withdrawn during the reaction, after the separation of phases, they have been chelated with 1.5 ml of a same iron concentration solution.

Both the product obtained from the synthesis #4 and the samples chelated have been dried in oven. The synthesis #4 has yielded in 117 grams of powder.

Products chromatographic analysis

From the analysis of product of synthesis #4, it has been obtained the following chromatogram:

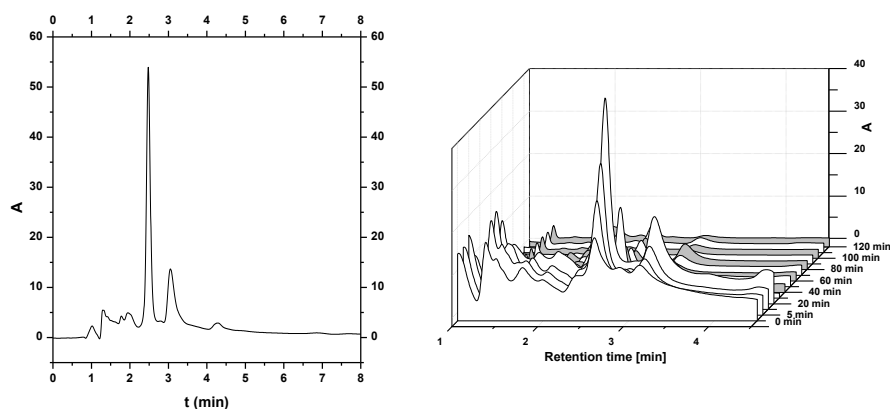


Figure 50. Chromatogram of product of the synthesis #4 (on the left) and absorbance vs retention time with the proceed of the synthesis #4 reaction time (on the right)

The chromatogram of product of the synthesis #4 shows the presence of both the isomers of *o,o*-EDDHA/ Fe^{3+} , *rac-o,o*-EDDHA/ Fe^{3+} and *meso-o,o*-EDDHA/ Fe^{3+} , which have been detected respectively after 2.5 minutes and 3.1 minutes. Considering the height of peaks, the product seems to be poor in the chelated iron content but nevertheless, the chromatogram is clean and only few amount of impurities have been detected at the beginning of the analysis.

By the 3D plot of Figure 49 is not possible to individuate a trend in the reaction evolution, due to the not similar powder amount analyzed for each withdrawn samples, but the negative results of synthesis #4 is also confirmed by the yield evolution with time:

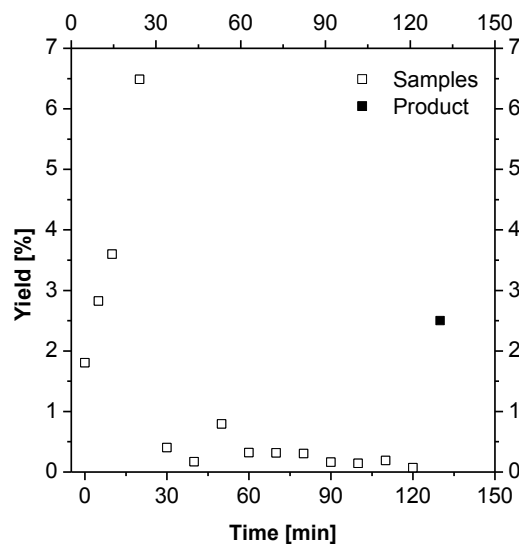


Figure 51. Yield evolution with reaction time for the synthesis #4

From the Figure 51 is clear the negative results of synthesis #4 according to the proposed protocol. In fact, in the first 20 minutes of reaction, the yield evolution seems to retrace the trend already highlighted in the previous described syntheses, but the proceeding of reaction shows a very poor reaction yield with a final yield of 2.5% (the empty square) with a fraction of o,o-EDDHA/Fe³⁺ of 0.18%. Obviously this protocol cannot represent an efficient milestone of an industrial process but these syntheses have been necessary to confirm that an excess of phenol is necessary to ensure a correct development of the reaction.

5.1.2 Synthesis #5

The synthesis #5 has been performed feeding the phenol in stoichiometric amount and the excess of organic solvents with one-third of phenol and two-third of toluene, so that the molar ratios of reagents have been:

Phenol: Toluene: Ethyl.: NaOH: Glyoxylic acid = 1.5: 2.4: 0.15: 0.3: 0.3

The reagents amounts fed is showed in the following table:

Table 19. Reagents fed for the synthesis #5

	Mole	Weight [g]	Volume [ml]
Phenol	1.5	141.165	131.93
Toluene	2.4	221.136	255.649
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			446.747

* 50% w/w water solution

The reaction has been carried out at $75\pm 5^\circ\text{C}$ for 120 minutes and at the end of reaction time the reactor has been cooled down and the extraction of phases has been performed. In the same way of syntheses #4, the volume of toluene used for the extraction phases has been decreased, respect to the volume used in the synthesis #3, of the quantity already dosed with the reagents:

Table 20. Solvents volumes used for the extraction phase of synthesis #5

Washing		Volume [ml]
1° washing	Water	500
	Toluene	500
2° washing	Water	100
	Toluene	300

Also in this case as toluene of the 2nd washing has been used a recycled toluene of the second washing of the others syntheses.

For the samples drawn from the reactor, the extraction of phases has been realized with 2.8 ml of water and 4.8 ml of toluene.

The water phase has been transferred in the second reactor and has been chelated with an iron (III) nitrate nonahydrate solution with and excess of 20% respect to the theoretical reaction yield of 60%:

Table 21. Chelating solution composition of synthesis #5

	Weight [g]
Water	200
Iron (III) nitrate nonahydrate	43.62

During the chelation 10 ml of sodium hydroxide 50% w/w water solution have been manually added to rapidly lead the pH in the range of set point value and in fact in 3 minutes and 36 seconds the pH has been stabilized at 7.3 value and, by the action of the peristaltic pump of caustic soda has been reached the 7.5 pH.

The samples taken during the reaction, after the separation of phases, have been chelated with 1.5 ml of a same iron concentration solution at about the 7.5 pH.

After the drying, from the synthesis #5 has been obtained 121.63 grams of powder.

Products chromatographic analysis

From the analysis of product obtained applying the protocol of the synthesis #5, it has been obtained the following chromatogram:

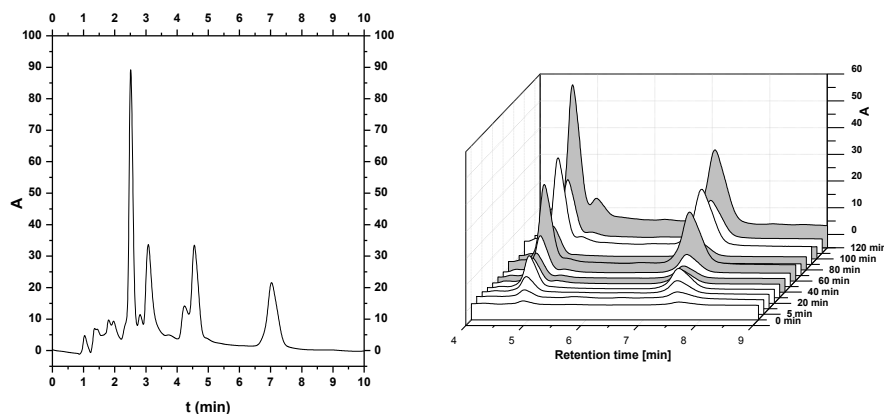


Figure 52. Chromatogram of product of the synthesis #5 (on the left) and absorbance vs retention time with the proceed of the synthesis #5 reaction time (on the right)

From the chromatogram is possible to identify the two peaks relative to the racemic and meso isomers of o,o-EDDHA/Fe³⁺, respectively at 4.5 and 7 minutes, and some other peaks, in the first three minutes of

analysis, are detected. Probably the peak at 3 minutes represents the o,p-EDDHA/Fe³⁺ while the other peaks indicate the presence of compounds of small dimension which are detected in the first stage of the analytical determination.

From the analysis of the sample withdrawn during the reaction can be obtained some interesting kinetics information, in fact also in this case, observing the 3D plot of Figure 52 between the retention time on the abscissa, the signal intensity on the ordinate, and the reaction time on the z axis is possible to have an idea of the evolution of products formation with the reaction time. Observing the increasing of peaks height it seems that the quantity of product in the reacting mixture increases in the time and this growth characterized the first 60 minutes of reaction; later, the difference between the areas of the peaks decreased, indicating that the reaction probably is ended (the chromatogram of the last sample is due to the quantity of analyzed powder).

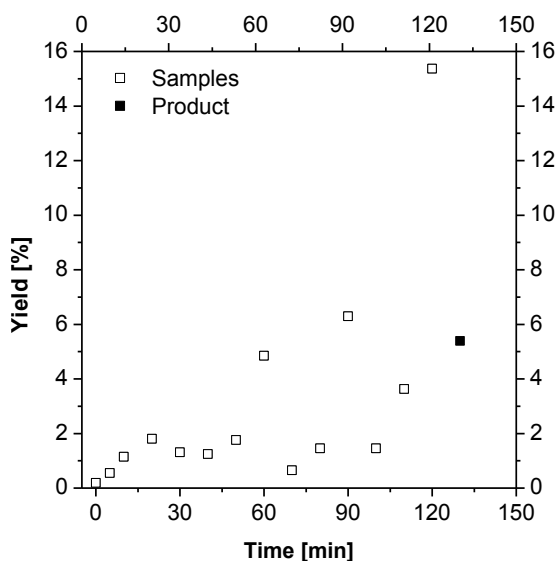


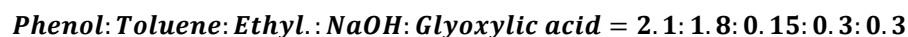
Figure 53. Yield evolution with reaction time for the synthesis #5

By the calculation of the reaction yield and the ortho-ortho chelated iron fraction, it is possible to evaluate the yield evolution with time. In the Figure 53, the reaction yield evolution is shown and by the graph is not possible to individuate a relation of the yield evolution with time and also the separation and the chelation of the withdrawn samples have been affected by experimental problem.

However the synthesis #5 results in a yield of 5.4% and o,o-EDDHA/Fe³⁺ amount of 0.3%, values which do not justify a further experimental analysis on the protocol developed for the synthesis #5.

5.1.3 Synthesis #6

The synthesis #6 has been performed feeding the phenol in stoichiometric amount and the excess of organic solvents has been ensured with phenol and toluene in equal part, so that the molar ratios of reagents have been:



The reagents amounts fed is shown in the following table:

Table 22. Reagents fed for the synthesis #6

	Mole	Weight [g]	Volume [ml]
Phenol	2.1	184.702	197.631
Toluene	1.8	165.852	191.736
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			435.607

* 50% w/w water solution

The reaction has been carried out at 75±5°C for 120 minutes and at the end of reaction time, after the cooling of reactor, the extraction of phases has been performed considering the reduction of toluene volume used in the syntheses #3 with the same criteria of synthesis #4 and #5:

Table 23. Solvents volumes used for the extraction phases of synthesis #6

Washing		Volume [ml]
1° washing	Water	500
	Toluene	550
2° washing	Water	100
	Toluene	300

In the same way of syntheses #4 and #5, as toluene of the 2nd washing has been used a recycled toluene of the second washing of the others syntheses.

For the samples drawn from the reactor, the extraction of phases have been realized with 2.8 ml of water and 4.8 ml of toluene.

The water phase has been transferred in the second vessel and has been chelated with an iron (III) nitrate nonahydrate solution with an excess of 20% respect the theoretical reaction yield of 60%:

Table 24. Chelating solution composition of synthesis #6

	Weight [g]
Water	200
Iron (III) nitrate nonahydrate	43.62

To rapidly reach the set point pH value, during the chelation 6 ml of sodium hydroxide 50% w/w water solution have been added manually and in 2 minutes and half the pH has been stabilized on the 7.5 value.

The samples taken during the reaction, after the separation of phases, have been chelated with 1.5 ml of a same iron concentration solution at about the 7.5 pH.

After the drying, from the synthesis #6 has been obtained 74.5 grams of product.

Products chromatographic analysis

From the analysis of products of syntheses #6, it has been obtained the following chromatogram:

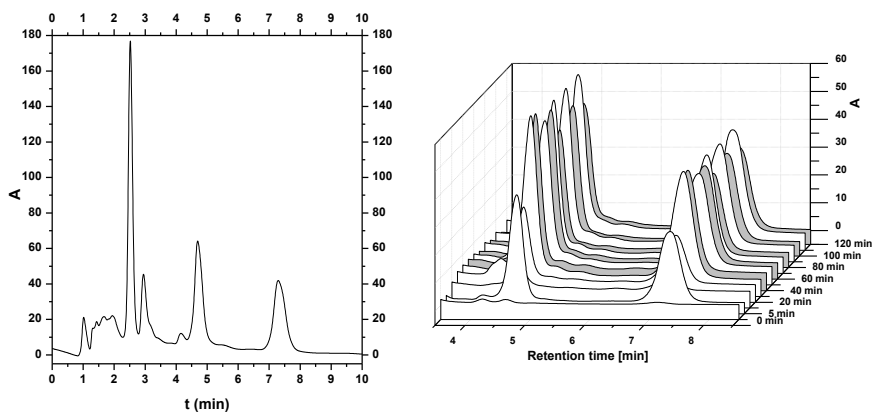


Figure 54. Chromatogram of product of the synthesis #6 (on the left) and absorbance vs retention time with the proceed of the synthesis #6 reaction time (on the left)

The chromatogram of product of the synthesis #6 highlights the presence of both the diastereoisomeric form of o,o -EDDHA/ Fe^{3+} which have been detected after 4.3 minutes, the racemic- o,o -EDDHA/ Fe^{3+} , and 7.3 minutes, the meso- o,o -EDDHA/ Fe^{3+} . From the chromatogram is also possible to individuate the peak at 3 minutes, probably related to the presence of o,p -EDDHA/ Fe^{3+} , and other peaks at the beginning of detection time which confirmed the presence of impurities.

From the analysis of the sample withdrawn during the reaction (Figure 54) is possible to have an idea of the evolution of products formation with the reaction time and in fact from the chromatograms evolution is possible to quantify the evolution of the reaction by the increasing of the peaks areas, relative to the racemic and meso o,o -EDDHA/ Fe^{3+} . Comparing the areas of the peaks, the reaction pattern is rather clear: at the beginning of the reaction the product in the reacting mixture significantly increases with the time, till about the 40th minute of reaction; later, the difference between the areas of the peaks are negligible, indicating that the reaction probably is ended.

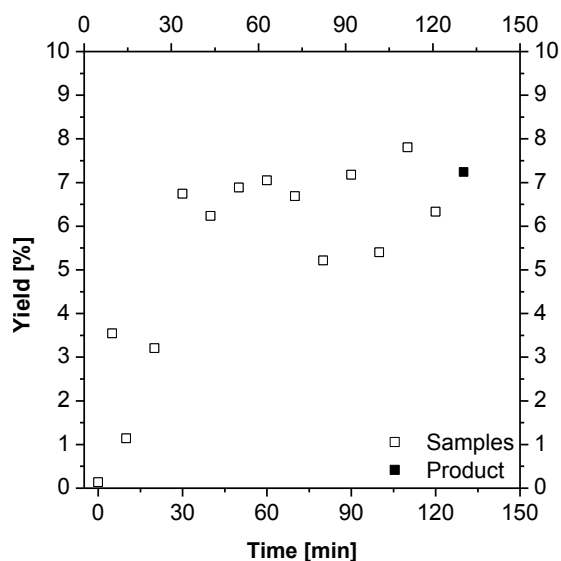


Figure 55. Yield evolution with reaction time for the synthesis #6

Observing the yield evolution with time of Figure 55 is possible to obtain the desired information about the reaction development: in fact, the reaction evolves rapidly in the first 30 minutes, later, till the 60 minutes, the yield continues to increase but after the first hour of reaction is clear the achievement of a plateau value, to confirm that the reaction time suggested by the literature is highly overestimated.

However the synthesis #6 have resulted in a yield of 7.5% and o,o-EDDHA/Fe³⁺ amount of 0.8%, results which do not justify a further experimental work on the protocol developed.

5.1.4 Synthesis #7

The synthesis #7 has been performed feeding the phenol in stoichiometric amount and saturating the excess of organic solvents with two-third of phenol and one-third of toluene, so that the molar ratios of reagents have been:

Phenol: Toluene: Ethyl: NaOH: Glyoxylic acid = 2.7: 1.2: 0.15: 0.3: 0.3

The reagents amounts fed is showed in the following table:

Table 25. Reagents fed for the synthesis #7

	Mole	Weight [g]	Volume [ml]
Phenol	2.7	254.097	237.474
Toluene	1.2	127.824	110.568
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			424.467

* 50% w/w water solution

The reaction has been carried out at $75\pm 5^\circ\text{C}$ for 80 minutes and at the end of reaction time, after the cooling of reactor, the extraction phases has been performed considering the reduction of toluene volume used in the syntheses #3 with the same criteria of syntheses #4, #5, #6:

Table 26. Solvents volumes used for the extraction phases of synthesis #7

Washing		Volume [ml]
1° washing	Water	500
	Toluene	620
2° washing	Water	100
	Toluene	300

In the same way of previous syntheses, as toluene of the 2nd washing has been used a recycled toluene of the second washing of the others syntheses.

For the samples drawn from the reactor, the extraction of phases have been realized with 2.8 ml of water and 4 ml of toluene.

The water phase has been transferred in the second vessel and has been chelated with an iron (III) nitrate nonahydrate solution with an excess of 20% respect the theoretical reaction yield of 60%:

Table 27. Chelating solution composition of synthesis #7

	Weight [g]
Water	200
Iron (III) nitrate nonahydrate	43.62

After the adding of iron salt solution, 6 ml of sodium hydroxide 50% w/w have been added to faster reach the set point 7.5 pH value, which it has been reached in 2 minutes and 20 seconds.

The samples taken during the reaction, after the separation of phases, have been chelated with 1.5 ml of a same iron concentration solution at about the 7.5 pH.

After the drying, from the synthesis #7 has been obtained 86.3 grams of powder.

Products chromatographic analysis

From the analysis of product of synthesis #7, it has been obtained the following chromatogram:

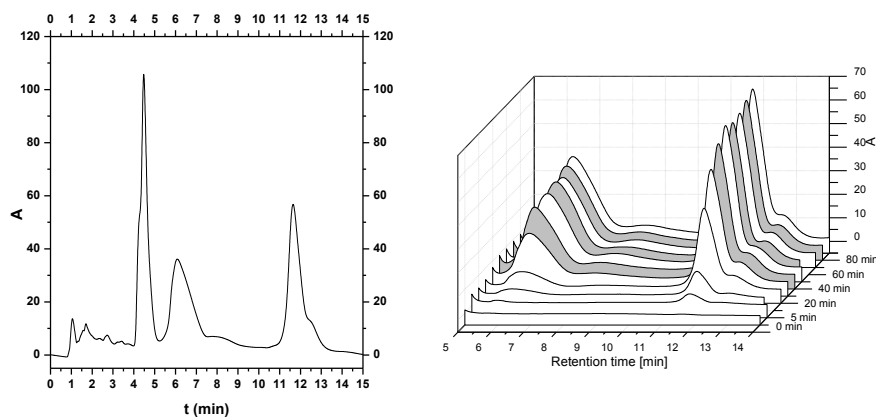


Figure 56. Chromatogram of product of the synthesis #7 (on the left) and absorbance vs retention time with the proceed of the synthesis #7 reaction time (on the right)

The chromatogram of product of the synthesis #7 shows the presence of both, racemic and meso, diastereoisomeric forms of o,o-EDDHA/Fe³⁺ which have been detected after 6 and 11.6 minutes. From the chromatogram is also possible to individuate the peak at 4.5 minutes, probably related to the presence of o,p-EDDHA/Fe³⁺ isomer.

Comparing the areas of the peaks, the reaction pattern is immediate visible: at the beginning of the reaction the product in the reacting mixture significantly increases with the time, in the first 50 minutes minute of reaction; later, the difference between the areas of the peaks are negligible, indicating that the reaction probably is ended.

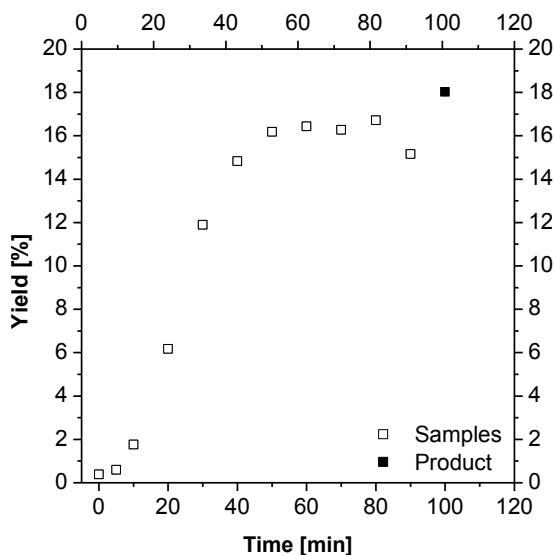


Figure 57. Yield evolution with reaction time for the synthesis #7

Observing the yield evolution with time, the desired information about the reaction development are obtained. As possible to note from the Figure 57, the reaction evolves rapidly in the first 40 minutes. Later, till the 60 minutes, the yield continues to increase but after the first hour of reaction is clear the achievement of a plateau value, indicating that the reaction is probably ended.

This evolution confirms that the reaction time is much lower than that indicated in literature, which means that, according with this analysis, a lot of time can be saved. In particular, considering the experiences of previous syntheses the reaction time has been already reduced and in fact, the synthesis #7 has been carried out for 90 minutes instead of the classic 120 minutes. At the end of the process, a very interesting average yield of 19% and an o,o-EDDHA/Fe³⁺ amount of 1.7% have been obtained.

5.2 Feed ratio analysis results

The screening about the feeding conditions has permitted to evaluate the effects of phenol partial substitution in the reaction mixture, with the consequent reduction of toluene used during the extraction of phases, on the synthesis results. In the following graph are summarized the obtained results in terms of reaction yield and ortho-ortho chelated fraction.

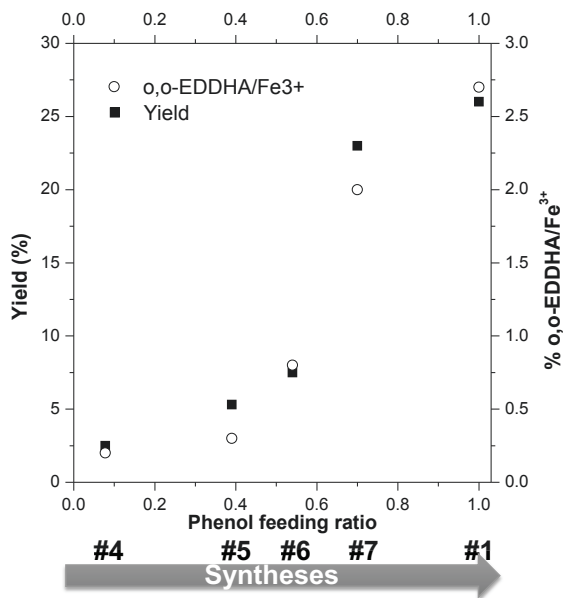


Figure 58. Phenol feed ratio investigated

Table 28. Feed ratio investigation results

Protocol	Reaction yield [%]	o,o-EDDHA/Fe ³⁺ fraction [%]
Synthesis #4	2.5	0.18
Synthesis #5	5.4	0.3
Synthesis #6	7.5	0.8
Synthesis #7	19	1.7
Syntheses #1	27	2.6

From Figure 58 and Table 28 is clear as the syntheses #4, #5 and #6 do not represent an industrial applicable process to optimize, due to the low reaction yield and ortho-ortho chelated iron fraction. Instead the synthesis #7, compared with the reference syntheses #1, with the 19% of reaction yield and 1.7% of o,o-EDDHA/Fe³⁺ represents the best compromise between the syntheses results and the reduction of solvents involved in the process.

The feed ratio tested for the synthesis #7 has represented the milestone of further process optimization and it has been selected as starting point to reach the aims of the work.

Moreover, based on the analysis of reaction evolution it is clear as 60 minutes are enough to the reaction end and for this reason the reaction time of following syntheses can be further decrease respect the reaction time adopted till now.

5.3 Extraction phases optimization

Based on the conditions in which synthesis #7 have been carried out, the reduction of solvents volumes used during the phases separation has been tested.

The extraction of phases at the end of reaction step is a fundamental part of process because it permits to recover the water phase which has to be chelated, deprived as possible of the organic solvents, and by the action on the volumes of solvents used to promote the phases separation is possible to regulate not only the demixing of phases present in the reactor but also the costs of process in terms of volumes used, post processing costs, and time. With the fixed global goal to create an optimized and environmentally friendly process and based on the observation that during the previous syntheses performed, the organic phase obtained from the second extraction is completely uncontaminated and it has represented by pure toluene, the second washing of water phase with toluene has been eliminated and moreover the volume of toluene dosed during the first extraction has been slightly decreased.

5.3.1 Syntheses #8

The reagents have been fed according to the molar ratios tested in the syntheses previous described:

Table 29. Reagents fed for the syntheses #8

	Mole	Weight [g]	Volume [ml]
Phenol	2.7	254.097	237.474
Toluene	1.2	127.824	110.568
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			424.467

* 50% w/w water solution

In this case, according to the reasons explained in the paragraph before, one only washing with water and toluene has been performed and, to evaluate the effects of volumes reduction only, the toluene used has been fresh solvent.

Table 30. Solvents volumes used for the extraction phases of syntheses #8

Washing		Volume [ml]
1° washing	Water	400
	Toluene	600

Also for the syntheses #8 the kinetic analysis has been performed and the 2 ml samples, drawn from the reactor, have been extracted with 1.9 ml of water and 3.1 ml of toluene.

The water phase obtained from the one step extraction phases has been collected and transferred in the second reactor ready to be chelated. In this case, understood the relation between the salt excess, the quantity of obtained product powder and the fraction of iron chelated as ortho-ortho, it has been chosen the salt excess of 20% respect the 60% of theoretical reaction yield, in order to obtained more powder to be used for the further applications:

Table 31. Chelating solution composition of syntheses #8

	Weight [g]
Water	50
Iron (III) nitrate nonahydrate	43.632

Contemporary to the iron salt solution addition, 1.5 ml of sodium hydroxide 50% w/w have been manually added to swiftly raise the pH in the set point range and by the combined action with the peristaltic pumps the set point pH value has been reached in 8 minutes

The samples drawn during the reaction, after the separation of phases, have been chelated with 0.5 ml of a same iron concentration solution at about the 7.5 pH.

Because of the collecting of oil and water phases, after the separation of phases during the extraction phases, is a manual process and it can be affect by human error, three times the syntheses #8 has been reproduced and for the batches have been obtained:

- batch#1 77.3 g of powder;
- batch#2 104.11 g of powder;
- batch#3 101.2 g of powder.

Products chromatographic analysis

From the analysis of products of syntheses #8, it has been obtained the following characteristic chromatogram:

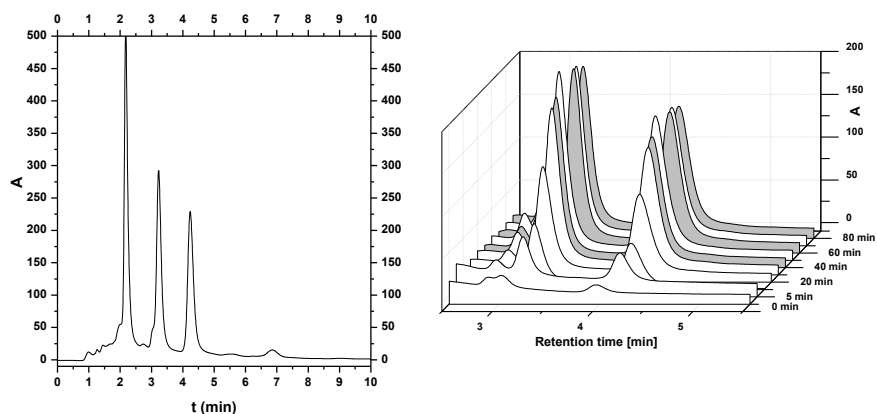


Figure 59. Chromatogram of product of the syntheses #8 (on the right) and absorbance vs retention time with the proceed of the syntheses #8 reaction time (on the left)

The chromatogram of product of the syntheses #8 shows the presence of both the isomers of EDDHA, which are detected at 3.25 minutes, the *rac*-*o,o*-EDDHA/ Fe^{3+} , and 4.2 minutes, the *meso*-*o,o*-EDDHA/ Fe^{3+} . The peak at about 2 minutes probably represents the *o,p*-EDDHA/ Fe^{3+} in a chromatogram very clean for a chelated iron.

From the analysis of the sample withdrawn during the reaction can be obtained some interesting kinetics information. Observing the 3D of Figure 59 is possible have an idea of the evolution of products formation with the reaction time. In fact, comparing the area of peaks, is clear as at the beginning of the reaction the product formation drastically increases in the reacting mixture, than the product in the reacting mixture slowly increases and after the first hour of reaction, the difference between the areas of the peaks is negligible, probably indicating the reaction end.

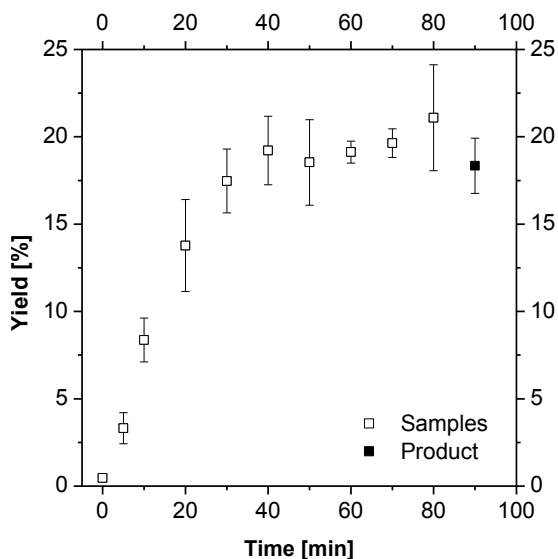


Figure 60.Yield evolution with reaction time for the syntheses #8

The Figure 60 confirms as after 60 minutes a plateau value of reaction yield is reached and this clearly indicates the reaction end.

The syntheses #8 have resulted in an average reaction yield of about 19%, and an o,o-EDDHA/ Fe^{3+} fraction of 1.8% have been obtained.

5.4 Recycle of organic solvents

The results of experimental work explained till now have permitted to perfectly understand the process, the parameters on which is possible to act to regulate the process results and the way to follow to reach the aims of work.

In fact, by the results presented it have been possible:

- to verify the feasibility of substitution of chlorinated substances with not chlorinated substances;
- to identify the best organic solvents feed ratio, with a partial reduction of phenol excess and its substitution with toluene;
- to confirm the possibility to reduce the volumes of solvents used to promote the phases separation during the extraction phases and the number of extraction steps;

Based on these observation and focused on the final aims of work, the syntheses #9 have been realized considering the necessity to minimize the use of organic solvents, an important item both in terms of costs

and environmental impact, and for this reason a recycling of organic solvents has been realized.

5.4.1 Synthesis #9

By the synthesis #7 it has been possible to find the best molar ratio organic solvent/ethylenediamine, and in particular the 3.6 mole of organic solvent respect the 0.15 moles of limiting reactants have been saturated with 2.4s mole of phenol (66.7% of solvent fed) and 1.2 moles of toluene (33.3% of solvent fed). According to this result, a mixture phenol/toluene 70/30% w/w has been prepared (with a density of $1.0085 \frac{g}{cm^3}$ and molecular weight of $93.519 \frac{g}{mol}$) and fed to the reactor to saturate the organic solvents excess while the stoichiometric phenol for the reaction has been fed separately. Moreover, considering that for the extraction of phases is required a water not miscible solvent and considering that neither toluene nor phenol are water miscible, the mixture with same composition has been used for the phases extraction.

All these efforts are finalized to save the use of organic solvent and with this aim well fixed in the research work, a series of 5 batches have been performed, following the same reaction scheme and recycling the phenol/toluene mixture during the reaction and the phases extraction steps.

Obviously, the fresh phenol/toluene mixture has been used for the first batch and the first samples of kinetic analysis of the battery.

Based on these considerations, the reaction has been carried out feeding the reagents according the following table:

Table 32. Reagents fed for the syntheses #9

	Mole	Weight [g]	Volume [ml]
Phenol	2.4	225.864	333.596
Toluene	1.2	110.568	
Fresh phenol	0.3	28.233	26
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			405.056

* 50% w/w water solution

At the end of feeding of reactants ($45\pm 2^\circ\text{C}$), the reactor temperature has been increased till $75\pm 5^\circ\text{C}$ and the reaction has been carried out for 80 minutes. At the end of reaction time, the reactor has been cooled down by the circulation of cold water in the reactor jacket and by the addition of solvents for the phases extraction. Considering the molar ratio between the not water miscible solvents fed to promote the phases separation and the limiting reactants, equal to 37, 5.5 moles of mixture phenol/toluene have to be dosed to reach the desired separation of phases present in the reactor.

Table 33. Solvents volumes used for the extraction phases of syntheses #9

Washing		Volume [ml]
1° washing	Water	400
	Phenol/toluene mixture	515

The oil phase extracted from the reactor is essentially composed by the mixture phenol/toluene, acting as solvent both in the reaction and separation phases, the unreacted phenol, and a very small amount of water phase which is not soluble in organic solvent. This organic phase is stored in a buffer tank from where, when necessary in the process, the organic mixture is taken. This recycle of organic solvents is the key passage to create a closed loop of their employment.

The reaction kinetic evolution has been analyzed drawing 2 ml samples and filling the test tube with 1.9 ml of water and 4 ml of phenol/toluene mixture, to promote the extraction phases.

The water phase obtained from the phases separation has been chelated with the iron nitrate solution considering an iron excess of 20% respect the theoretical reaction yield of 60%. Moreover, the water volume used to dissolve the salt has been drastically reduced from 200 ml to 50 ml inasmuch the water does not participate to the chelation reaction but it acts as solvent only:

Table 34. Chelating solution composition of syntheses #9

	Weight [g]
Water	50
Iron (III) nitrate nonahydrate	43.632

Contemporary to the iron salt solution addition, 2 ml of sodium hydroxide 50% w/w water solution have been manually added to rapidly increase the pH up to the set point range and by the combined action of the peristaltic pumps the set point pH value has been reached in about 7 minutes

The withdrawn samples, after the separation of phases, have been chelated with 0.5 ml of a same iron concentration solution at about the 7.5 pH.

Five batches have been produced with the above described process, always recycling the phenol/toluene mixture, to test the feasibility of the recycling of the organic solvents and the obtained results have been very interesting. From each batch has been obtained:

- batch#1 75.2 g of powder;
- batch#2 115.64 g of powder;
- batch#3 87.77 g of powder;
- batch#4 108.55 g of powder;
- batch#5 90.4 g of powder.

Products chromatographic analysis

From the analysis of products of each batch of syntheses #9, the following characteristic chromatograms have been obtained:

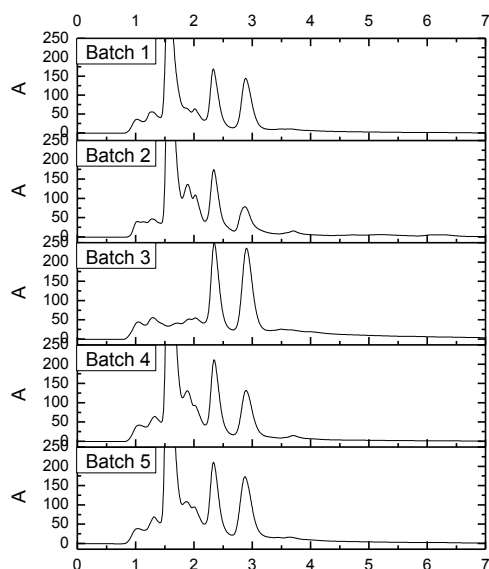


Figure 61. Chromatograms of products of the batches of syntheses #9

The chromatograms of products of the batches of syntheses #9 confirm the presence of both the isomeric form of EDDHA, which are detected at about 2.4 minutes, the *rac*-*o,o*-EDDHA/Fe³⁺, and 2.9 minutes, the *meso*-*o,o*-EDDHA/Fe³⁺. The peak at about 1.5 minutes probably represents the *o,p*-EDDHA/Fe³⁺ in a chromatogram very clean for a chelated iron.

Also for the batches of syntheses #9 the reaction evolution has been determined by the analysis of the sample withdrawn during the reaction. Considering the results of kinetic analysis of previous syntheses, the reaction time has been further decreased to 70 minutes. The average values are showed in the following figure:

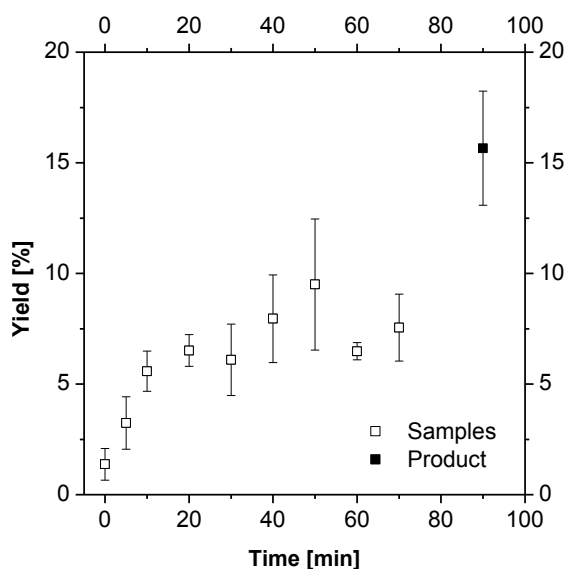


Figure 62. Yield evolution with reaction time of syntheses #9

In the Figure 62 are represented the average values of yield of the five batches with the reaction time and, considering the previous already explained difficult in the perfect reproducibility of extraction of phases and the chelation of withdrawn samples, the vertical error bars are shown. Nevertheless, the reaction evolution is clear and retraces the trend observed for the syntheses analyzed before and in fact, the product amount rapidly increase, in the reacting mixture, in the first stage of reaction after that the yield continues to increase till the 50 minutes. Unfortunately the points related to 60 and 70 minutes are not useful in these evaluation because problems in the separation of phases and in the chelation of samples occurred.

An interesting aspect of the recycle of the organic solvent mixture, in the feeding of reactants and as solvent to promote the phases separation, is the progressive darkening of the mixture and in fact from the third batch of syntheses #9 the color of organic solvent mixture and the water phase containing the EDDHA produced is the same.

The results, in terms of reaction yield and ortho-ortho chelated iron fraction are showed in the following table:

Table 35. Results of batches of syntheses #9

Batch number	Reaction yield [%]	o,o-EDDHA/Fe ³⁺ fraction [%]
n° 1	9.47	1
n° 2	12.18	1
n° 3	18.32	1.7
n° 4	16.53	1.2
n° 5	15.61	1.4

The average reaction yield obtained from the syntheses #9 is slightly smaller than 18%-20% obtained in the previous syntheses but the syntheses #9 have been realized with a closed loop of use of the organic solvent, a real revolution in the production of chelated iron. The syntheses #9 prove the feasibility of more environment friendly process, with a drastic reduction of solvents use and with a reduction of costs, also, due to the less use of substances and post processing operations.

Moreover, the protocol of syntheses #9 can be further optimized and in particular from the process point of view. In fact, as explained before, after the third cycle of using of phenol-toluene mixture, the color of the mixture is comparable to the color of water phase in the reactor and the identification of separation phases line is very difficult with a consequent possibility of error in the phase recovery (loss a part of water phase or collecting a part of oil phase with water phase). To prevent this kind of error, a density sensor can help in the exact recognition, and extraction from reactor, of water and oil phase.

**Results and discussion:
chelation optimization**

6.1 Iron salt excess evaluation

As noted during the previous syntheses performed, acting on the iron salt excess is possible to regulate the powders amount obtained from the process and the fraction of chelated iron as ortho-ortho isomer.

Till now, except for the syntheses #1 and #2, an excess of 20% respect to the theoretical yield of 60% has been adopted. Considering the yields of reactions performed, the theoretical yield reached in the Petree et al. patent [14] never has been reached and in fact the best result obtained is a reaction yield of about the 25%.

Based on this results, two syntheses have been performed reducing the iron salt excess from the 20% on the 60% of yield to the 20% on the 25% of reaction yield in order to optimize the iron salt consumption and to reduce the final product cost.

In order to evaluate the only effects of iron salt excess in the chelating solution, the protocol of syntheses #8 have been reproduced modifying only the chelating solution.

6.1.1 Syntheses #10

The reagents have been fed according the molar ratios fixed for the syntheses #7, and their amounts are showed in the following table:

Table 36. Reagents fed for the syntheses #10

	Mole	Weight [g]	Volume [ml]
Phenol	2.7	254.097	237.474
Toluene	1.2	127.824	110.568
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			424.467

* 50% w/w water solution

The reaction has been carried out at $75\pm 5^\circ\text{C}$ for 90 minutes and at the end of this stage, the reactor temperature has been cooled down and the separation phases step has been performed. According to the protocol of syntheses #8, the volumes of solvents fed for the extraction of phases have been:

Table 37. Solvents volumes used for the extraction phases of syntheses #10

Washing		Volume [ml]
1° washing	Water	400
	Toluene	600

The toluene used for the extraction has been fresh solvent, in order to eliminate eventual contaminations that can influence the syntheses results.

Also for these syntheses, the evaluation of kinetic evolution has been performed and in particular the 2 ml samples, drawn from the reactor, have been extracted with 1.9 ml of water and 4 ml of toluene.

The water phase, obtained from the extraction phases, has been transferred in the second reactor and chelated with the iron (III) nitrate solution with an iron excess of 20% respect the yield considered of 25%:

Table 38. Chelating solution composition of syntheses #10

	Weight [g]
Water	200
Iron (III) nitrate nonahydrate	18.18

Also in this case, after the adding of the iron solution the pH has fallen down and, to rapidly increase the pH in the range of set point, 2.5 ml of sodium hydroxide 50% w/w water solution have been added. By the action of peristaltic pumps, which completes the regulation to the 7.5 pH value, the pH has been reached in 2 minutes and half.

The samples taken during the reaction, after the separation of phases, have been chelated with 1 ml of a same iron concentration solution at about the 7.5 pH.

From the scratching of product after the drying have been obtained:

- batch#1 77.4 g of powder;
- batch#2 65 g of powder.

Products chromatographic analysis

The chromatogram of products of syntheses #10 is following showed:

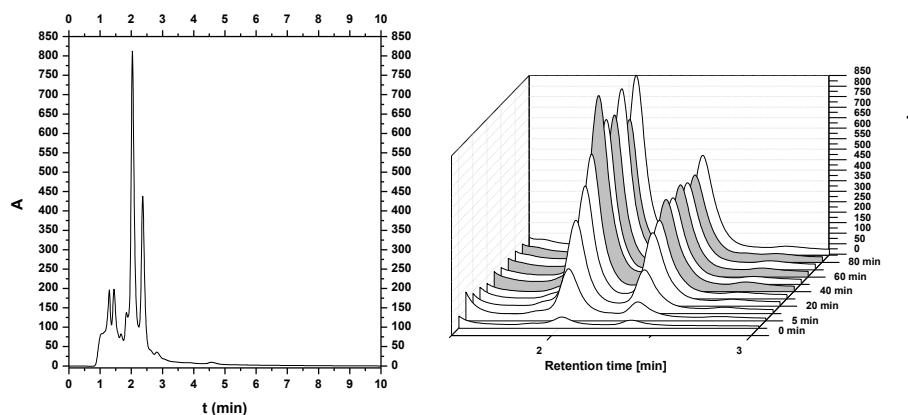


Figure 63. Chromatogram of product of the syntheses #10 (on the left) and absorbance vs retention time with the proceed of the syntheses #10 reaction time (on the right)

The Figure 63 highlights the presence of both, racemic and meso, diastereoisomeric form of *o,o*-EDDHA/ Fe^{3+} which have been detected in the first three minutes of analysis and in particular, respectively, after 2 and 2.4 minutes. The chromatogram is clean enough and the height of EDDHA peaks permits, also, to flatten the peaks relative to the impurities.

From the analysis of the sample withdrawn during the reaction can be obtained some interesting kinetics information, in fact by the 3D plot of Figure 63 is possible have an idea of the evolution of products formation with the reaction time. Comparing the areas of the peaks, the reaction pattern is immediate clear: at the beginning of the reaction, the product formation in the reacting mixture significantly increases with the time, in the first 50-60 minutes of reaction; later, the difference between the areas of the peaks decrease drastically, indicating probably the reaction end.

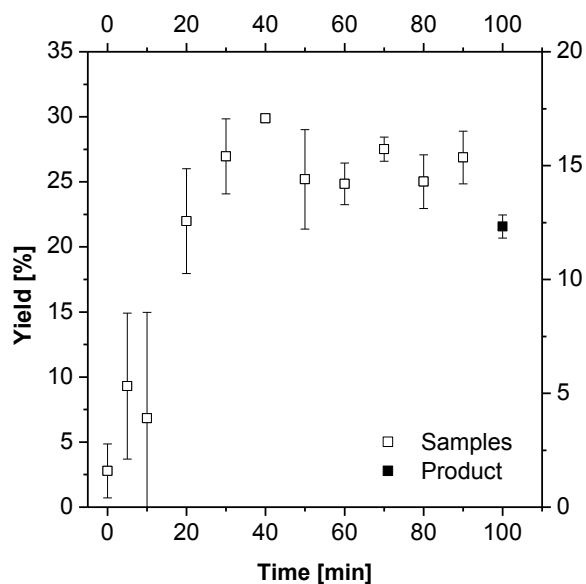


Figure 64. Yield evolution with reaction time for the syntheses #10

By the yield evolution with time the desired information about the reaction development are obtained. As possible to note from the Figure 64, the reaction evolution retraces the trend already highlighted in the previous syntheses and in fact the reaction evolves rapidly in the first 40 minutes, later, till the 60 minutes, the yield continues to increase slowly but after the first hour of reaction a plateau value is reached, indicating the ending of the reaction. This is a further confirmation that the reaction time considered by the literature is overestimated.

The synthesis #8 results in a yield of 22%, very close to the reference synthesis results, and an o,o-EDDHA/ Fe^{3+} amount of 2.8% have been obtained.

The syntheses #10 have permitted to evaluate the effects of iron salt excess on the ortho ortho fraction in the produced chelated iron and the relationship between the salt excess in the chelating solution and the amount of product powder.

6.2 Iron salt change

The recycling of organic solvents has been successfully tested and it has been showed the feasibility of closed loop of solvents use. The process described for the syntheses #9 is surely of a great interest for the industrial application because, respect to the literature processes, it

permits an economical optimization, by the realization of closed loop for the use of organic solvents, by the reduction of volumes of solvents used with a consequent reduction of the post processing costs and time, and by the reduction of the necessary reaction time, and it permits to work with a more environmental compatible process. Nevertheless the optimization of process needs of another important step: the testing of a cheaper iron salt than iron (III) nitrate nonahydrate. The iron nitrate is a perfect iron salt to be used for the production of iron chelated because it permits to supply the iron in form of iron chelated, the fraction chelated with the EDDHA, and of soluble iron, the fraction which is not chelated, but thanks to the presence of nitrate group also the nitrogen nitric is present in the product and available for the plants. Unfortunately, the iron (III) nitrate nonahydrate is very expensive, 2.7 euros for kilo, and this has an important impact on the final cost of product. For this reason the testing of a cheaper iron salt has been necessary.

After a technical-economic analysis, the iron (III) sulfate has been chosen to be tested, considering its cost (0.50 euros for kilo of product) and its chemical-physical characteristics.

Two series of syntheses have been performed following the reaction pathway of the syntheses #9 with the substitution of the iron salt, the first one of 5 batches to reproduce exactly the syntheses #9 and to compare the results, and the second one of 9 batches to evaluate the effects of the continued organic solvents recycling on the reaction results and on the process.

Obviously the starting batch of each series has been obtained using fresh organic solvents mixture.

6.2.1 Syntheses #11

According to the syntheses #9, the reagents have been fed according to the following table:

Table 39. Reagents fed for the syntheses #11

	Mole	Weight [g]	Volume [ml]
Phenol	2.4	225.864	333.596
Toluene	1.2	110.568	
Fresh phenol	0.3	28.233	26
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			405.056

* 50% w/w water solution

When the reaction is ended the reactor has been cooled down and the solvents to promote the phases separation have been dosed:

Table 40. Solvents volumes used for the extraction phases of syntheses #11

Washing		Volume [ml]
1° washing	Water	400
	Phenol/toluene mixture	515

Also for the syntheses #11, during the reaction the 2 ml samples have been drawn from the reactor and placed in a test tube filled with 1.9 ml of water and 4 ml of phenol/toluene mixture, to promote the extraction phases.

After the separation of phases present in the reactor, the water phase extracted from the bottom of reactor has been transferred in the second vessel and chelated with an iron (III) sulfate concentrated solution, considering an iron excess of 20% respect to the theoretical yield of 60%:

Table 41. Chelating solution composition of syntheses #11

	Weight [g]
Water	50
Iron (III) sulfate	21

The regulation of pH, as done in the previous syntheses described, has been performed with an initial manual dosing of caustic soda (1.5 ml of sodium hydroxide 50% w/w water solution) and with the fine pH adjustment realized by the caustic soda peristaltic pump till to reach the set point pH value. The necessary time to reach the final pH has been of about 8 minutes because the response of system to the addition of chemicals has been slower than EDDHA solution chelated with iron nitrate.

The samples taken from the reactor have been deprived of oil phase and chelated with 0.4 ml of iron (III) sulfate solution at the same concentration of that used for the chelation of batch product.

The product obtained from the chelation reaction has been dried in oven and quantitatively recovered. From the five batches of the first series have been obtained:

- batch#1 60.89 g of powder;
- batch#2 83.7 g of powder;
- batch#3 74.9 g of powder;
- batch#4 78 g of powder;
- batch#5 74.6 g of powder.

The nine batches of the second series have yielded in:

- batch#1 57.2 g of powder;
- batch#2 82.7 g of powder;
- batch#3 71.0 g of powder;
- batch#4 80.0 g of powder;
- batch#5 80.5 g of powder;
- batch#6 75.6 g of powder;
- batch#7 65.1 g of powder;
- batch#8 94.5 g of powder;
- batch#9 70.3 g of powder.

Products chromatographic analysis

From the analysis of products obtained from each batch of the first battery of synthesis #11, the following characteristic chromatograms have been obtained:

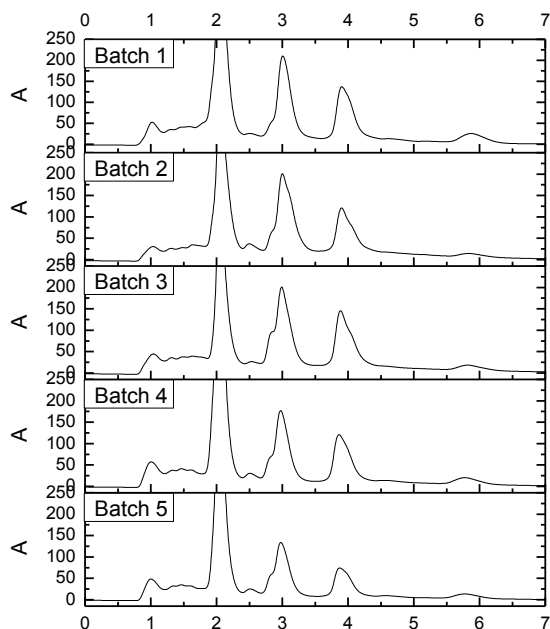


Figure 65. Chromatograms of products of batches of the first battery of syntheses #11

From the chromatograms of Figure 65 is clear the presence of *o,o*-EDDHA/Fe³⁺ isomers which are detected at 3 minutes, the racemic form of *o,o*-EDDHA chelated iron, and about 4 minutes, the *meso* form. Moreover, the chromatograms highlights the important presence of *o,p*-EDDHA/Fe³⁺ by the peaks at about 2 minutes and it is possible to note as all the products chromatograms are very clean, despite the recycling of solvents could suggest the obtaining of a product with more impurities.

Very interesting is also the analysis of characteristic chromatograms of products obtained from each batch of second battery of syntheses according the protocol of synthesis #11:

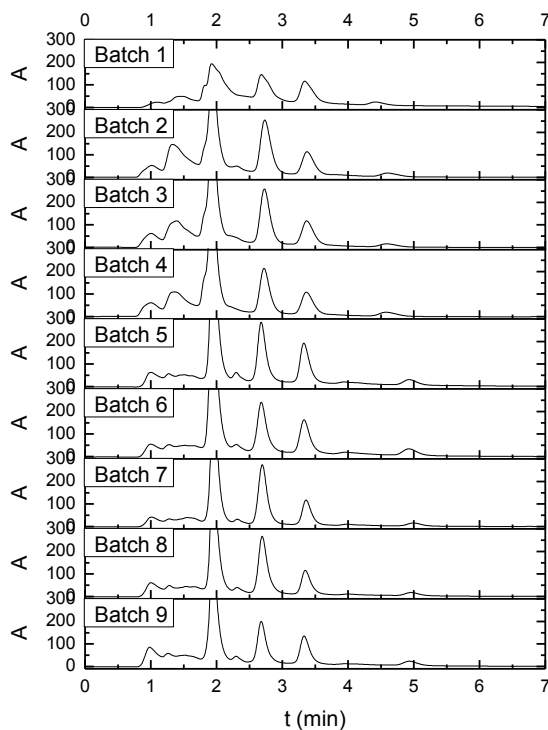


Figure 66. Chromatograms of products of batches of the second battery of syntheses #11

The second series of nine syntheses also confirms the reproducibility of products characteristics and in fact all samples present both the isomer of *o,o*-EDDHA/Fe³⁺ detected respectively at about 2.7 minutes, the *rac*-*o,o*-EDDHA/Fe³⁺, and at 3.4 minutes, the *meso*-*o,o*-EDDHA/Fe³⁺. The peak at about 2 minutes represents the *o,p*-EDDHA/Fe³⁺ probably.

To validate once again the kinetic results obtained till now, also for the batteries of syntheses #11 the reaction evolution has been analyzed by the withdrawing of samples during the reactions.

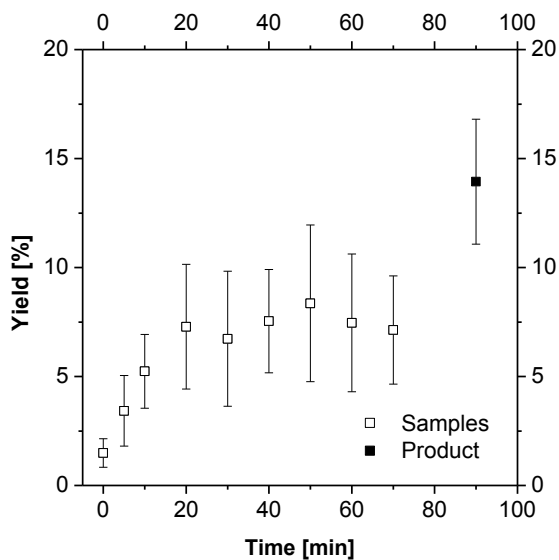


Figure 67. Yield evolution with reaction time of the first battery of syntheses #11

The kinetic analysis confirms the reaction evolution identified in the previous described syntheses in which the product formation proceeds rapidly for the first 40 minute, after that the reaction continues slowly till the first hour of reaction from when the product amount in the reacting mixture does not increase, indicating the reaction end. The low value of reaction yield are affected by the last batch results which have resulted the worse in terms of reaction yield and ortho-ortho EDDHA fraction.

In the Figure 68 the results of kinetic analysis for the second series of syntheses #11 are showed:

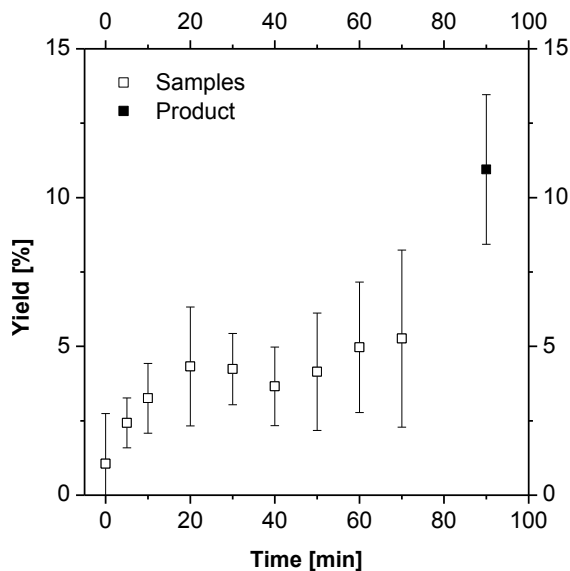


Figure 68. Yield evolution with reaction time of the second battery of syntheses #11

The battery of nine batches confirm the reaction pattern found and certify definitely as a reaction time of 60 minutes is enough to the reaction end. This permits to absolute certainty state that the literature reaction time is greatly overestimated.

Regarding the low reaction yield value, this has been affected by the low reaction results of the first batch of battery, due to the characteristics of paste at the outlet of chelation reactor, which do not permit a perfect recovery of product, and to the seventh batch for which the 7.5 pH value, during the chelation, has been reached in a longer time than other syntheses.

Moreover, in the evaluation of results shall be considered as the progressive darkening of organic solvents mixture has made more difficult the collecting of separated phases, due to the difficult to identify the exact line of phases separation and consequently the correct volumes recovered for each phase.

The results, in terms of reaction yield and ortho-ortho chelated iron fraction are showed in the following tables:

Table 42. Results of batches of the first battery of syntheses #11

Batch number	Reaction yield [%]	o,o-EDDHA/Fe ³⁺ fraction [%]
n° 1	12	1.7
n° 2	16.5	1.8
n° 3	16.8	1.8
n° 4	13.8	1.5
n° 5	10	1.2

The average reaction yield of 14% obtained from the first battery of syntheses #11 is smaller than 18%-20% obtained in the syntheses #7, #8, #9 and #10 but the closed loop in the organic solvents use and the reduction of solvents volume and the consequently post processing operations represent a very interesting compromise for the industrial practice. Moreover, it has to be taken in account that this has been the first experiment carried out using the iron (III) sulfate instead of the iron (III) nitrate nonahydrate and for this reason a process optimization, under this point of view, can be performed.

The batches of the second battery of syntheses have guaranteed the following results:

Table 43. Results of batches of the second battery of syntheses #11

Batch number	Reaction yield [%]	o,o-EDDHA/Fe ³⁺ fraction [%]
n° 1	6	1
n° 2	13.2	1.4
n° 3	12	1.4
n° 4	11.2	1.2
n° 5	11.8	1.2
n° 6	11.3	1.2
n° 7	9.4	1.21
n° 8	13.44	1.4
n° 9	11	1.2

From the Table 43 is clear as the first and the seventh batches have been the worse in terms of reaction yield but it is also clearly visible

the perfect reproducibility of the syntheses results both in terms of final reaction yield and o,o-EDDHA/Fe³⁺ fraction. This highlights the important experimental work done in the standardization of the several phases of the production process of o,o-EDDHA/Fe³⁺ which have guarantee a reproducibility of process and their results.

At this point, it is necessary focus the attention to the increase, as possible, of the reaction yield and the ortho-ortho fraction, in particular, to the achievement of the best process configuration.

Moreover, as already explained for the synthesis #11, an optimization, by using a density sensor, of the step of recovery of oil and water phases can be performed. This can ensure the collecting of correct volume of water phase obtained, to be chelated, (with an impact on the reaction yield) and avoid the pollution of oil phase, to be recycled in the successive process.

6.3 Characterization of iron (III) sulfates

The syntheses results, both in terms of process yield and ortho-ortho chelated iron amount, are also related to the quality characteristics of the iron salt used. The company Fertenia SrL have supplied two different samples of iron (III) sulfates available on the market, which have been characterized and compared with the iron (III) sulfate technical grade supplied by Sigma-Aldrich.

The characterization of the three different iron (III) sulfate formulations has been carried out by the spectrophotometric analysis, to determine the absorption of light in the UV-VIS range, and by the thermogravimetric (TGA) analysis, to determine the compounds present in the samples.

The three formulation analyzed have been:

- Sample #1: iron (III) sulfate supplied by Sigma-Aldrich;
- Sample #2: iron (III) sulfate supplied by Fertenia SrL;
- Sample #3: iron (III) sulfate supplied by Fertenia SrL.

6.3.1 Spectrophotometric analysis

To carry out the spectrophotometric analysis, a known amount of iron salt has been dissolved in 50 ml of distilled water, in order to produce an iron solution at known concentration.

Each iron solution has been analyzed investigating the whole spectrum, from 110 nm to 200 nm.

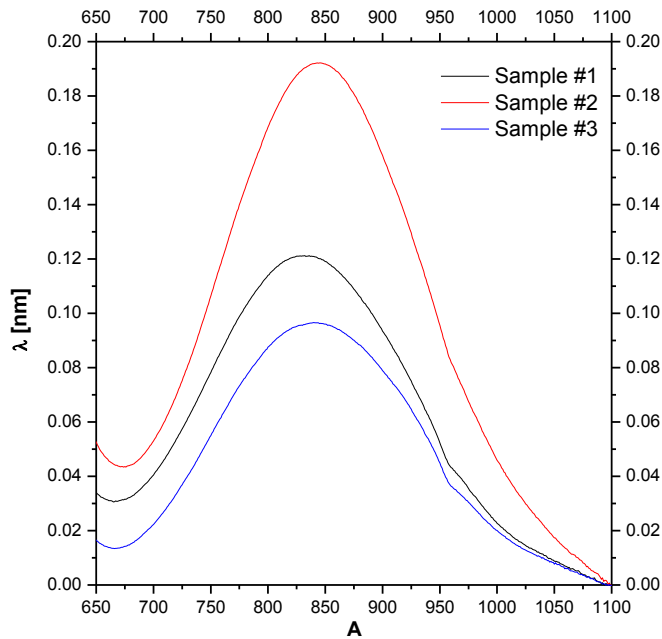


Figure 69. Spectrum of analyzed samples

As it is possible to observe from the spectrum of Figure 69, where is reported only the section comprised between 1100 nm and 650 nm, the peak of absorption for the three solution is at the wavelength of about 840 nm.

Table 44. Spectrophotometric analysis results

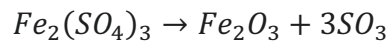
	c [g/L]	A [-]	A/c [L/g]
Sample #1	1.75	0.12	0.07
Sample #2	1.60	0.19	0.12
Sample #3	1.66	0.1	0.06

The Table 44 shows the results of spectrophotometric analysis carried out on the iron solution characterized, where c is the concentration of iron solution analyzed in grams for liter, A is the absorbance of analyzed solution and, according to the Lambert-Beer law is possible to calculate the calibration constant, k , as the ratio between A and c .

6.3.2 Thermogravimetric analysis (TGA)

The thermogravimetric analysis is an analytical technique where is recorded the continuous mass variation of a sample, placed in controlled atmosphere, in function of temperature and time. The TGA results are expressed in graph where in abscissa there is the time and/or the temperature and on the ordinates there is the weight loss as absolute value or as percentage.

The reference of iron (III) sulfate TGA is the decomposition reaction of salt:



in fact by the removal of SO_3 is possible to quantify the iron sulfate present in the sample.

6.3.2.1 TGA of sample #1

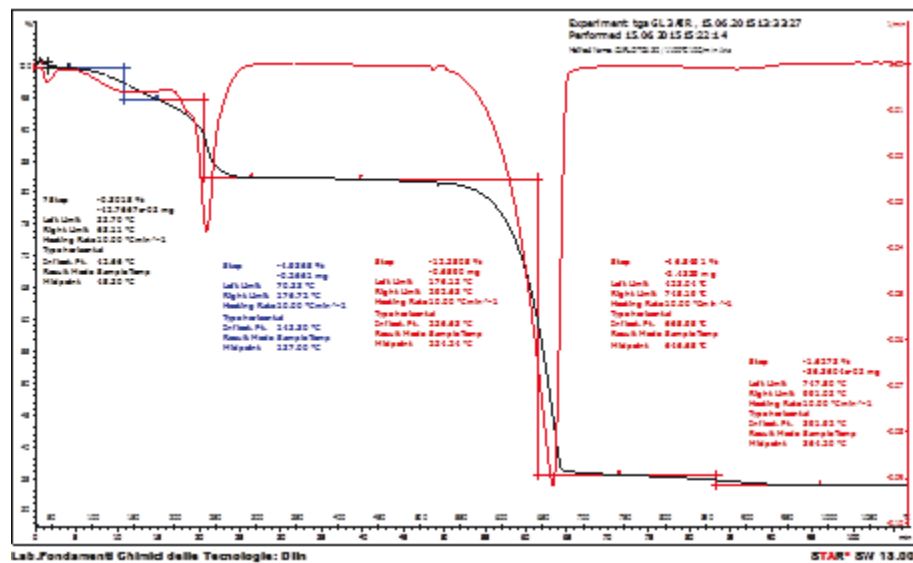


Figure 70. TGA of sample #1

The sample #1, as shown in Figure 70, is characterized by two weight loss (black curve) which indicate the removal of volatile compounds and the removal of SO_3 , respectively. The residual part represents the Fe_2O_3 and the impurities that can be quantified by the before described decomposition reaction.

Considering 100 g of a sample #1, it is possible to calculate:

Considering a calculation basis of 100 grams of sample #2, it is possible to obtain:

Table 46. TGA analysis results for the sample #2

	Molecular weight [g/mol]	n [mol]	m [g]
H ₂ O and volatiles	18	1.667	30.000
SO ₃	80	0.425	34.000
Fe ₂ (SO ₄) ₃	400	0.142	56.667
Fe ₂ O ₃	160	0.142	22.667
Impurities			13.333
TOT			100

From the Table 46 is possible to note as the sample #2 contains the 57% of iron sulfate, the 13% of impurities and hydration ratio of 11.8.

6.3.2.3 TGA of sample #3

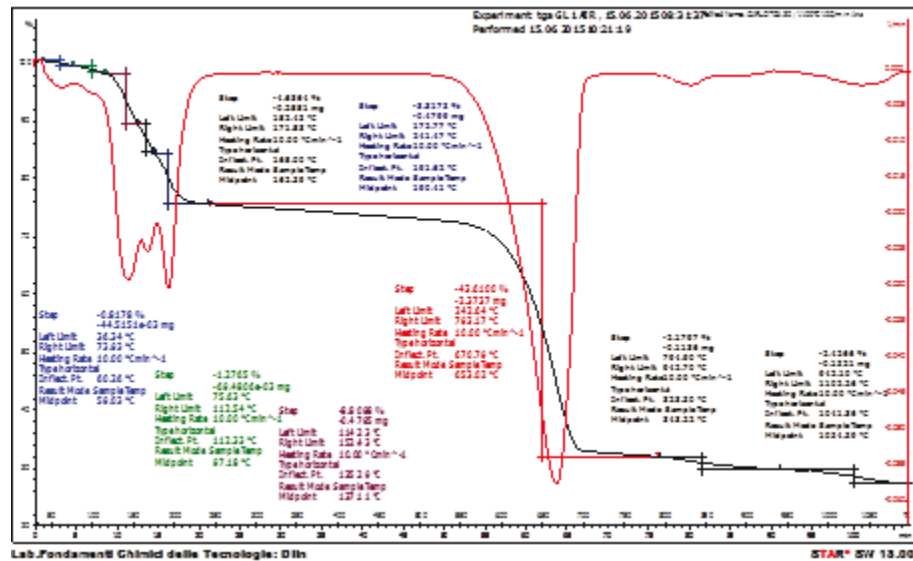


Figure 72. TGA of sample #3

From the Figure 72 is clear as the TGA of sample #3 is characterized by two weight loss indicating, respectively, the volatile compounds

(72%) and the SO₃ removal (33%). The residual part represents the Fe₂O₃ and the impurities.

Considering a calculation basis of 100 grams of sample #3, is possible to calculate:

Table 47. TGA analysis results for the sample #3

	Molecular weight [g/mol]	n [mol]	m [g]
H ₂ O and volatiles	18	1.556	28.000
SO ₃	80	0.488	39.000
Fe ₂ (SO ₄) ₃	400	0.163	65.000
Fe ₂ O ₃	160	0.163	26.000
Impurities			7.000
TOT			100

The sample #2 contains the 65% of iron sulfate, the 7% of impurities and an hydration ratio of 9.6%.

6.3.3 Iron (III) sulfate characterization results

Based on the results of the analytical determination, the sample #1 is the purer product with lower moisture level. The sample #2 and #3, the products supplied by Fertenia SrL which are available on the market, are characterized by an high level of impurities and hydration ratio. Regarding the Fe₂(SO₄)₃, the sample #3, with 65%, is better than sample #2, with 56.7%, which it is, also, negatively characterized by the 13.3% of impurity level.

The results of syntheses realized with the iron (III) sulfate have been affected by the quality of salt used, in fact using an iron salt with high impurity level and low iron (III) sulfate fraction, the produced EDDHA is not completely chelated and both, the process yield and the ortho-ortho chelated fraction are negatively affected.

6.4 Optimization of iron (III) sulfate use

Based on the results of iron (III) salt characterization, the iron (III) sulfate supplied by Sigma Aldrich has been selected to perform a

cycle of syntheses to verify the results can be obtained using this iron salt.

6.4.1 Syntheses #12

The reagents have been fed according to the protocol of syntheses #9, as showed in the following table:

Table 48. Reagents fed for the syntheses #12

	Mole	Weight [g]	Volume [ml]
Phenol	2.4	225.864	333.596
Toluene	1.2	110.568	
Fresh phenol	0.3	28.233	26
Ethylenediamine	0.15	9.015	10.017
NaOH	0.3	12	116*
Glyoxylic acid	0.3	22.212	33.152*
Reagents Volume (V_{reagents})			405.056

* 50% w/w water solution

When the reaction is ended the reactor has been cooled down and the solvents to promote the phases separation have been dosed:

Table 49. Solvents volumes used for the extraction phases of syntheses #12

Washing		Volume [ml]
1° washing	Water	400
	Phenol/toluene mixture	515

Known the kinetic reaction pattern, for the syntheses #12 the sample withdrawing has been not performed.

After the separation of phases present in the reactor, the water phase extracted from the bottom of reactor has been transferred in the second reactor and chelated with an iron (III) sulfate concentrated solution, considering an iron excess of 20% respect the theoretical yield of 60%:

Table 50. Chelating solution composition of syntheses #12

	Weight [g]
Water	50
Iron (III) sulfate	21

The regulation of pH, as done in the previous syntheses described, has been performed with an initial manual dosing of caustic soda (1.5 ml of sodium hydroxide 50% w/w water solution) and with the fine pH adjustment realized by the caustic soda peristaltic pump till to reach the set point pH value.

The product obtained from the chelation reaction has been dried in oven and quantitatively recovered. From the six batches of the cycle have been obtained:

- batch#1 64 g of powder;
- batch#2 79 g of powder;
- batch#3 72 g of powder;
- batch#4 81 g of powder;
- batch#5 74 g of powder;
- batch#6 75 g of powder.

6.4.2 Products chromatographic analysis

From the analysis HPLC analysis of products obtained from each batch of the syntheses #12, the following characteristic chromatograms have been obtained:

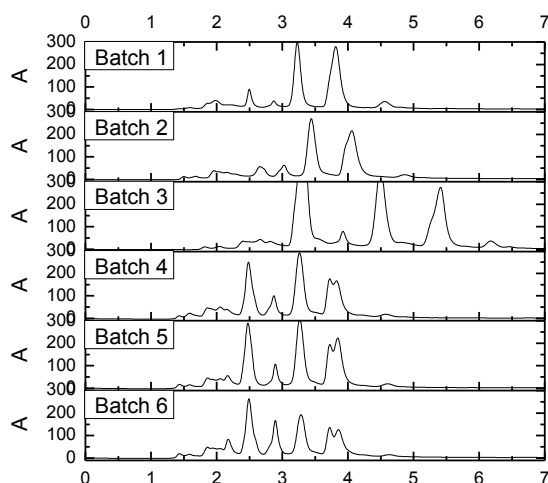


Figure 73. Chromatograms of products of batches of syntheses #12

From the chromatograms of Figure 73 is clear the presence of *o,o*-EDDHA/Fe³⁺ isomers which are detected at about 3 minutes, the racemic form of *o,o*-EDDHA chelated iron, and about 4 minutes, the meso form, instead for the batch 3 where the peaks are shifted of about 1.5 minutes; the *o,p*-EDDHA/Fe³⁺ presence is showed by the peaks at about 2.5 minutes and it is possible to note as all the products chromatograms are very clean, despite the recycling of solvents could suggest the obtaining of a product with more impurities. Based on the observations previous described about the darkening of organic phase with the recycle and the consequent difficulties to identify the separation line between organic and water phase, during the extraction phases, particular attention has been paid to this operation.

The results, in terms of reaction yield and ortho-ortho chelated iron fraction are showed in the following tables:

Table 51. Results of the syntheses #12

Batch number	Reaction yield [%]	<i>o,o</i> -EDDHA/Fe ³⁺ fraction [%]
n° 1	15	2
n° 2	19	2.1
n° 3	22	2.5
n° 4	18	1.9
n° 5	18.5	1.95
n° 6	13	1.2

The average reaction yield of syntheses #12 is 17.6%, comparable with the 18%-20% obtained in the syntheses #7, #8, #9. The closed loop in the organic solvents use, the reduction of solvents volume, with the consequent post processing operations reduction, and the iron salt change, with the replacement of expensive iron (III) nitrate nonahydrate with the cheaper iron (III) sulfate, represent a very interesting compromise for the industrial practice. The average ortho chelated iron fraction is 1.94% which represents a good results compared with the commercial product available on the market considering the strong process modifications proposed.

Based on this experimental screening about the iron salt, it is important highlights as the choice of the iron salt is fundamental to the obtainment of a good synthesis result. In fact, the substitution of iron (III) nitrate nonahydrate with the iron (III) sulfate can be performed, maintaining the positive synthesis, at the condition that the iron sulfate is of a good quality, with low impurity level and low moisture level. This is essential to guarantee the correct chelation with the iron, avoiding the chelation of competitive elements, and to avoid the production of chelated iron with unknown substances which can be even dangerous for the plants.

Chapter Seven

Results and discussion: product testing

Part of this work has been reported in:

P. Apicella, S. Cascone, G. Lamberti, “Optimization of chelating agents production process for agricultural administration of inorganic micronutrients”, proceedings of CHISA 2014, Prague, Czech Republic.

7.1 Comparison between syntheses products and commercial samples

Thanks to Fertenia SrL, the company which support this research project, has been possible the analysis of five commercial products and the comparison with the iron chelated produced according the protocols developed during the research work.

The samples have been analyzed dissolving about 60 mg of powder in 50 ml of alkali solution at concentration of 0.24 g/l of NaOH in water, in the same way of the synthetized products. In the following picture are showed the chromatograms of commercial products analyzed:

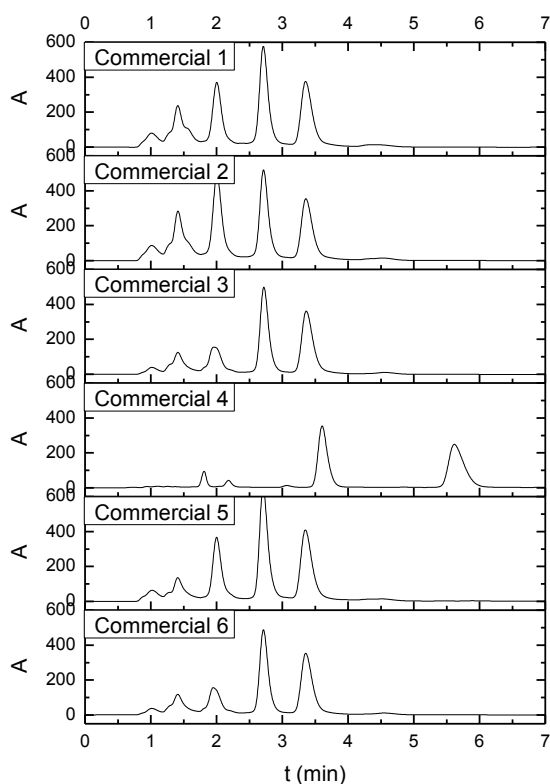


Figure 74. Chromatograms of commercial products

From the chromatograms of Figure 74 is clear the presence of both the isomers of o,o -EDDHA/ Fe^{3+} at 2.6 and 3.4 minutes for all the samples unless the commercial sample 5 where the peaks are detected at 3.6 and 5.6 minutes.

Comparing the chromatograms of the commercial products with those of products obtained from the syntheses described, and in particular with those of synthesis #12, the most interesting process from the industrial point of view, is clear as they are very similar. Both the kind of samples present a mixture of rac-o,o-EDDHA/Fe³⁺ and meso-o,o-EDDHA/Fe³⁺ as o,o-EDDHA/Fe⁺³ with an important fraction of iron chelated as o,p-EDDHA/Fe³⁺.

Regarding the ortho-ortho chelated iron fraction, in the following table are showed the results of the analytical determination and a comparison with those obtained by the protocols developed:

Table 52. ortho-ortho percentage fraction of commercial products

Sample	o,o-EDDHA/Fe³⁺ fraction [%]
Commercial product 1	3.7
Commercial product 2	3.5
Commercial product 3	3.4
Commercial product 4	3.2
Commercial product 5	4.1
Commercial product 6	3.3
Synthesis sample # 1	2.6
Synthesis sample # 2	1.2
Synthesis sample # 3	1.5
Synthesis sample # 4	0.2
Synthesis sample # 5	0.3
Synthesis sample # 6	0.8
Synthesis sample # 7	1.7
Synthesis sample # 8	1.8
Synthesis sample # 9	1.3
Synthesis sample # 10	2.8
Synthesis sample # 11	1.4
Synthesis sample # 12	2

As it possible to observe by the Table 52, the commercial products contain an average amount of o,o-EDDHA of 3.6%, with peak of 4%. These value are higher than those of products obtained by the protocols developed during the research work, where a value close to the 3% have been reached, but this difference is totally justified considering that the iron chelated are actually produced according well-established processes, completely optimized, whereas the protocols developed during the research work can be further optimized.

7.2 Image analysis

The image analysis, on the seedling of lettuce plants, has been performed taking every day a picture of samples to compare the life development of plants.

The “commercial” sample has been treated with the commercial product 2 in the previous paragraph analyzed (it has been chosen the product 2 because it has the ortho-ortho fraction closer the average value) whereas, the “T5A” sample has been treated with the product obtained from the synthesis #1, the first modified protocol of reference production process.

The concentration of chelated iron solution adopted has been 2 g of chelated iron powder for each liter of water, as suggested by the agricultural practice.

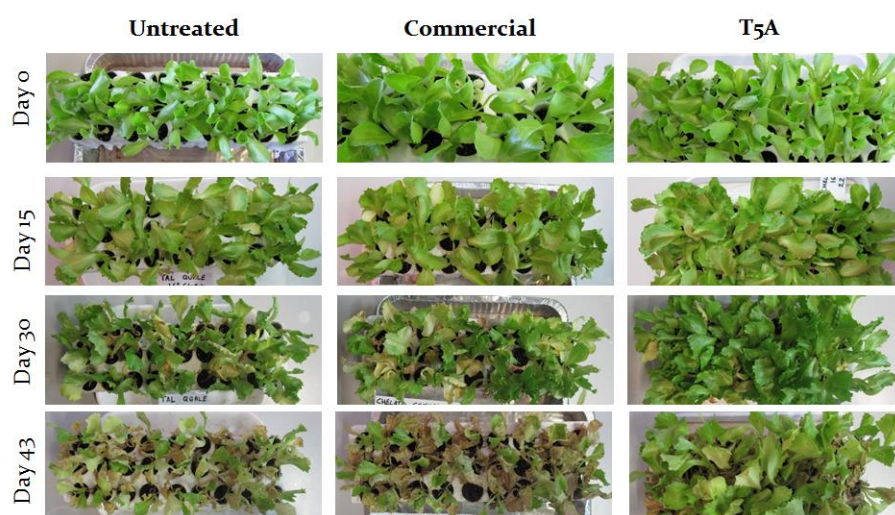


Figure 75. Image analysis

From the Figure 75 is clear, and also expected, that the seedling of untreated samples have been the first to died and after 30th day only few plants presented green leaves.

Regarding the comparison between the treated sample, it is immediate visible as the sample "T5A" is the best and after 43 days of cultivation, all the samples were considerably grew with a glossy green leaves. The "commercial" sample has exhibited a good development in the first 15 days of cultivation, with green leaves, after that a progressive yellowing of plants, with the iron chlorosis which has penetrated till the older leaves, can be noted, till the clear death of the lettuce plants showed in the picture.

By virtue of this, despite the commercial product 2 contains an higher amount of o,o-EDDHA/Fe³⁺, the product of synthesis #1 has been resulted more efficacious in the iron chlorosis therapy.

Chapter Eight

Design of industrial plant

The research project has been realized in collaboration with the company Fertenia SrL which is interested to the development and optimization of an innovative industrial process for the production of chelated iron for agricultural uses.

Moreover, the company is interested to the industrialization of the developed process and for this reason the design of the process and of the unit operations have been realized.

Fertenia SrL is a dynamic company located in via Luca Giordano 12, Bellizzi (Salerno), Italy. In the picture below is showed the maps of the factory and in the crossed square is highlighted the location of chelated iron production plant.

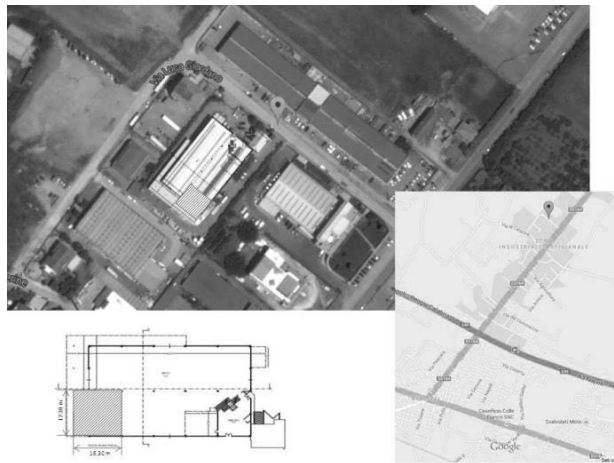


Figure 76. Fertenia SrL map

An internal area of the factory of 16.5 m x 17.5 m has been dedicated to the iron chelates plant and at the outside of the factory the storage tanks of chemicals will be located.

The process will be industrialized on the basis of the scheme of synthesis #12, where a reaction between ethylenediamine, phenol, glyoxylic acid and sodium hydroxide is performed in presence of phenol-toluene mixture, according the reaction condition successfully tuned during the experimental work previous described.

The preliminary design of the industrial plant has been started considering the productivity, $P_d = 3 \cdot 10^6 \frac{kg}{year}$, indicated by the company which means a daily production, considering 240 working days, of $12.5 \cdot 10^3$ kilos every day.

Moreover, considering an average reaction yield of 23%, an amount of 2.5% of o,o-EDDHA/Fe³⁺ and feed ratios developed during the experimental work, the mass balances have been positively solved and the preliminary design of the unit operation has been performed.

The detailed mass balances are reported in the Appendix A.

Based on the characteristics of the process and on the requirements of Fertenia SrL, a batch plant has been designed, considering one only unit of reaction/separation/chelation where the synthesis stages are performed.

8.1 Process design

The industrial process has been designed according to the results obtained from the experimental work, described before, in compliance with the company requirements.

The production time for each batch has been estimated to be about 3 hours and 35 minutes, excluded the drying time.

8.1.1 Phase 1: the reaction

Time: 28 minutes of reagents dosing + 60 minutes of reaction

The reaction phase can be divided in two sub-stages: the reagents feeding and the reaction phase. The phase of reactants feeding, of 28 minutes of duration, will provide the single dosage of the compounds in the following order:

- phenol-toluene mixture, 14 minutes;
- fresh phenol, 3 minutes;
- ethylenediamine, 4 minutes;
- sodium hydroxide 50% w/w water solution, 4 minutes;
- glyoxylic acid 50% w/w water solution, 3 minutes.

The volumes of reagents fed are listed in the following table.

Table 53. Volumes and pump flowrate of reagents fed

Reagents	Volume fed [m ³]	Pump flowrate [m ³ /h]
Phenol-toluene mix	8.33	36
Fresh phenol	0.1	2
Ethylenediamine	0.12	2
Sodium hydroxide 50% w/w solution	0.2	3.5
Glyoxylic acid 50% w/w solution	0.41	6

In this phase, the reactor temperature shall be maintained at 45±5°C. At the end of reactants feeding, the reactor temperature must be rapidly increased to 75±5°C and the reaction shall be carried out for 60 minutes.

8.1.2 Phase 2: phases extraction

Time: 29 minutes of solvents adding + 5 minutes of mixing

At the end of the reaction time, the reactor temperature shall be cooled down by the addition of the solvents, phenol-toluene mixture and water, to promote the separation of phases, and by the emptying of reactor jacket. Considering the high volumes of solvents to pump in the reactor, the times necessary are:

- water 15 minutes;
- phenol-toluene mixture 14 mixture.

The volumes of solvents dosed to promote the phases separation are expressed in the table below.

Table 54. Volumes and pump flowrate of solvents fed

Solvents	Volume fed [m ³]	Pump flowrate [m ³ /h]
Water	6.46	26
Phenol-toluene mix	8.4	36

During and after the addition of solvents, the mixer shall be active at high velocity (v_1), in order to guarantee the deep mixing of phases but

after 5 minutes, the motion shall be stopped and the settling of the phases have to take place.

8.1.3 Phase 3: settling and organic phase recovery

Time: 37 minutes of settling and organic phase recovery

During the settling phase, the system shall be completely stationary (mixer stopped and fluid in the jacket not circulating). At the end of phases settling, two phases will be clearly visible: the organic phase, the upper layer, with lower density than water, and the water phase, the lower layer, containing the chelating agent and water soluble unreacted reagents. The organic phase has to be extract and collected in the storage tank of phenol-toluene organic mixture, ready to be recycled for the later batches.

In order to ensure process flexibility and the possibility to extract the organic phase at different heights in the reactor, a periscope pipe driven by an electric motor has been provided.

8.1.4 Phase 4: chelation

Time: 40 minutes of iron salt solution adding, chelation and drain + 15 minutes of reactor cleaning

The water phase present in the reactor will be chelated with the iron salt concentrated solution prepared in the dedicated dissolver, and by the pH control system the pumps for the dosing of caustic and acid solutions will be activated based on the measured value and its difference with the 7.5 pH set point value. During this phase of process, it will be not necessary the circulation of fluid in the reactor jacket and the mixer will be active at medium velocity (v_2). The achievement of pH set point value must be performed in less time as possible and the system shall be mixed at 7.5 pH value for 30 minutes. After this time, the reactor will be discharged and the product collected in the dedicated tank, before to be send to the drying section.

Table 55. Iron salt concentrated solution

	Mass fed [kg]
Water	705.7
Iron salt	705.7

The concentrated iron solution pumping will go on for 5 minutes, the chelation for 30 minutes and the drain for 5 minutes reasonably.

After the discharging of product, the reactor will be cleaned, ready to restart the production cycle.

8.2 Design of the unit operations

8.2.1 Reactor/separator/chelator

A single pot unit only has been provided, with the function of reactor/separator/chelator, of volume of $V = 25 m^3$, able to guarantee the target productivity through one daily production cycle (batch), as requested by Fertenia SrL.

The unit has been designed as reverse section cone, in the lower part, with a cylinder part on the top, in order to guarantee the possibility to easily empty the reactor when necessary.

The unit has been equipped with a mixer with the helix suitable for high viscous paste, able to ensure at least three levels of motions: $v_1 = 100 rpm$, during the reaction and at the beginning of the extraction of phases, $v_2 = 30 rpm$, during the chelation and $v_3 = 10 rpm$, during the emptying of the reactor. Moreover, the mixer shall be able to guarantee the mixing of the different volumes present in the reactor depending on the production process phase, ensuring the mixing in the deeper part of the reactor also.

The reaction unit has been provided with a jacket for the circulation of fluids to the correct development of thermic history necessary to the process. During the reaction, of duration of 60 minutes, shall be ensured the reacting mixture temperature of $75 \pm 5^\circ C$ by the circulation of hot water in the jacket. At the end of reaction phase, the reacting mixture shall be cooled down and for this reason, the jacket has been equipped with a drain to discharge the hot water.

The reactor has been equipped with a temperature control system, necessary to check the thermal history developed in the reactor, and a pH monitoring and adjustment system especially, essential to the correct realization of chelation phase by the rapid pH adjustment on the 7.5 set point value.

The unit, moreover, has been provided with a variable height system for the extraction, from the top, of the oil phase obtained from the

extraction of phases. The system, assisted by the pump, must ensure low velocity of extracted organic phase to avoid the drag of water phase present in lower part of the reactor.

8.2.2 Reactor design

The reaction unit has been designed according to the process requirements and the constructions and transportation problems.

The diameter, D , has been fixed at 3 meter, limit to avoid special transport and the angle of conic part of 21.8° , for executive reason.

Based on this, to reach the target reactor volume of 25 m^3 , the cone height is 0.6 meter, with a volume of 1.41 m^3 , and the cylinder height is 2.3 m, with a volume of 23.59 m^3 .

8.2.3 Drier

The drier, E , has been design to treat the maximum volume of 9 tons of chelated iron solution (1.25 tons of iron chelates and 7.7 tons of water) every day, considering 8 daily working hours.

Collaborating with VOMM SpA, a company dedicated to the development and production of technologies to the drying, has been designed the drying section of industrial plant for the production of iron chelates.

The drying plant has been designed to treat $6500 \frac{\text{kg}}{\text{day}}$ of iron chelated solution, at 13-14% minimum of dry matter, to dry till the 98% of dry matter.

The drying is performed by the turbo-drying technology in which the creation and the progress, in turbulence condition, of a thin film of liquid against the internal wall of a cylindrical surface is performed. In this way, thanks to the high value of heat exchange coefficient, is reached an elevated thermal efficiency which ensures the drying of product minimizing the energy requirement.

The liquid is agitated in the turbo-drier by the action of turbine and a flow of hot air, in co-current with the product, is used to help the evacuation of steam produced during the drying.

The drying plant has been designed to work in a closed loop with condensation of evaporated. This water, at temperature of 80-85°C, can be used as sanitary water of company Fertenia SrL.

The drying plant is composed by the following components:

1. dosing of material section;
2. drying section;
3. separation section iron chelated powder/process gas and vapor;
4. discharge iron chelated powder section;
5. condensation and discharge of vapor section;
6. thermal recovery section;
7. utilities section.

According to the P&ID of drying section (**Errore. L'origine riferimento non è stata trovata.**) the drying process starts from the storage tank of iron chelated solution, TK1, from where the solution is pumped, by the mono screw pump MO1, to the drier ES1. At the outlet of drier, the powder is carried with the wet gas to the cyclone, C1, where the powder is separated and collected, from the bottom, ready to the packaging. The wet stream, from the top of C1, is first partially sent in a condensation column, CO1, and then used in the heat exchanger, E2, to recover energy as hot sanitary water.

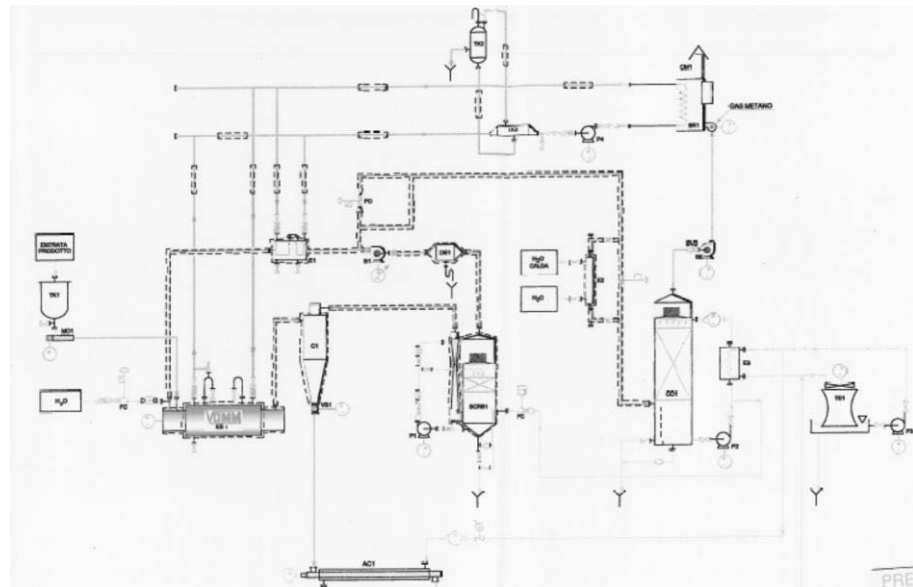


Figure 77. P&ID of drying plant

The designed plant is able to treat $812 \frac{kg}{h}$ of iron chelated solution, in order to reach the target productivity by 8 hours daily working hours.

By the designed drying plant is possible to evaporate $704 \frac{kg}{h}$ of water which can be used as process water during the productions of Fertenia SrL company.

8.3 Storage tanks

For each reagent dosed during the production process, a dedicated storage tank has been designed. The volume of storage tanks has been sized in such way to guarantee an adequate working capacity and each tank has been chosen of 20 m^3 . By this volume, in fact, has been ensured the following working period:

- TANK-01, phenol, 40 working days (excluded the phenol necessary to prepare the organic mixture);
- TANK-02, ethylenediamine, 160 working days ;
- TANK-03, glyoxylic acid 50% w/w water solution, 48 working days;
- TANK-04, sodium hydroxide 50% w/w water solution, 100 working days;

Moreover, considering the melting temperature of phenol of about 41°C , the storage tank of phenol shall be thermally traced or equipped with a jacket.

To store the phenol-toluene organic mixture necessary in the process, a storage tank, TANK-05, of 20 m^3 has been provided.

The water solution of chelated iron obtained from the process will be collected, before drying, in a storage tank, V-01, of 30 m^3 able to store about three batches.

To clean the reactor at the end of each batch and to recover the product from the wall of reactor, a cleaning system has been designed. This is composed by a toroidal pipe on which has been installed 28 spray balls ad hoc selected and arranged on the pipe, to ensure the maximum effect with the minimum water quantity (200-300 liters). The product recover will be collected in V-01 tank.

A dissolver, D-06, for the iron salt, of volume of 3 m^3 , has been designed to prepare the chelating solution necessary for each batch.

A small tank, of about 50 liters, of hydrochloric acid 37% water solution has been provided to the pH regulation during the chelation phase.

8.3.1 Ethylenediamine storage

To store in safety conditions the ethylenediamine (EDA), considering the physical and chemical characteristics, is necessary take some preventive measure.

The principal characteristics are:

- the best material for tanks, pipes and hoses is stainless steel (SS304 or 316 or equivalent);
- melting point temperature 11°C;
- flash point temperature 42°C;
- nitrogen blanketing is necessary in order to avoid formation of explosive mixture with air, discoloration and less good color stability, formation of amine carbonate;
- avoid emission of vapors containing allergenic amines;
- ensure a good ventilation.

Based on these, to store the EDA has been developed an ad hoc solution which consider the storage at 15-20°C in nitrogen blanketed tank.

8.3.1.1 Ethylenediamine storage system

In the Figure 78 is showed the scheme of system designed to store the ethylenediamine at 15-20°C.

The upper part of storage tank (that not occupied by the EDA) has been blanketed with nitrogen. The fumes released for the tank (ethylenediamine/nitrogen) have to pass in a fan/compressor, in a condenser, from which the flowrate EDA-nitrogen goes out at 15°C (a temperature which allows the ethylenediamine condensation) and finally in gas-liquid separator, which allows the ethylenediamine and nitrogen recovery (vent valve closed) recycled in the storage tank.

The most thorny phase is the loading of storage tank during which the fumes development is more probable. During this phase the vent valve to the atmosphere has to be open, in order to discharge nitrogen in atmosphere, avoiding overpressure in the tank and harmful emissions.

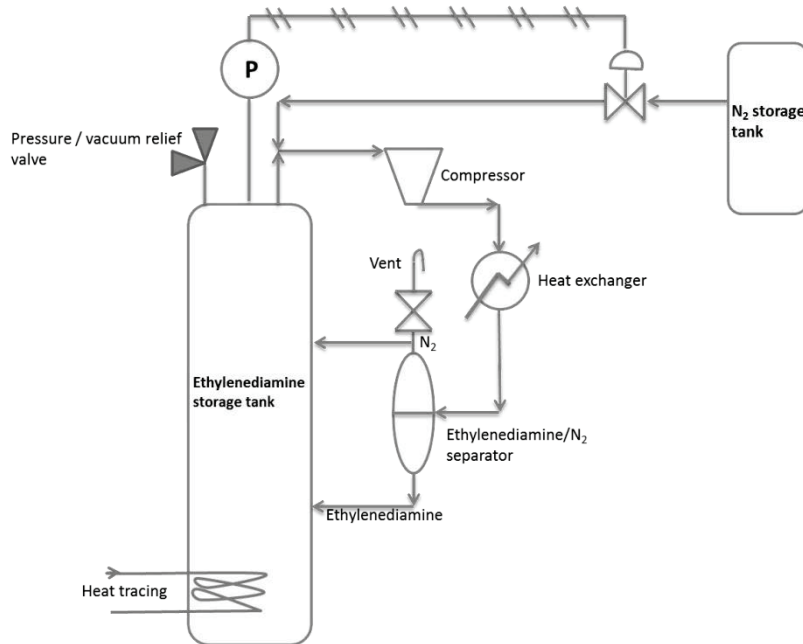


Figure 78. EDA storage system

Moreover, the tank has been provided with a pressure/relief valve to avoid eventual under and overpressure.

About the nitrogen consumption has to be consider that the nitrogen used to blanket the storage tank is recycled in the tank after the separation and for this reason the consumption is very low considering the necessity of pure nitrogen as make-up and to balance the ethylenediamine volume pumped to the process.

8.3.1.2 Internal storage tank temperature calculation

A preliminary passage to design the refrigeration system is the calculation of storage temperature of the nitrogen-EDA mixture.

According to the scheme showed in the Figure 79, the storage tank is at T_s temperature, radiated from a thermal flux q_s and exposed at thermal convective exchange with the external environment at T_a temperature.

The calculation has been realized considering the worse possible conditions, considering the summer season with a $q_s = 1100 \frac{W}{m^2}$, $h = 10 \frac{W}{m^2K}$ (no wind) and an external temperature $T_a = 28.5^\circ C$.

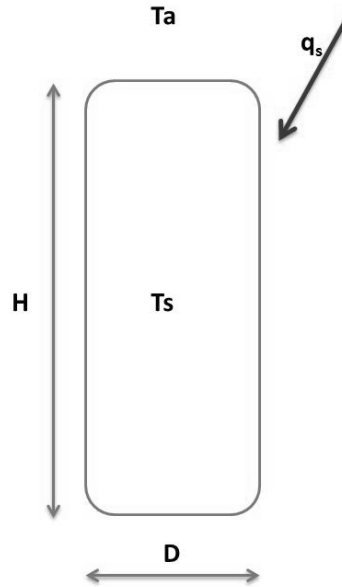


Figure 79. Scheme for T_s calculation

The thermic balance is:

$$A * h_s * \Delta T = \varepsilon * q_s * A_{exp}$$

Where A is the storage tank surface, A_{exp} is the surface exposed to the solar radiation, and considering the tank positioning $A_{exp} = \frac{A}{2}$. The ε coefficient represents the emissivity and is equal to 0.21 for stainless steel white painted storage tank.

Based on this:

$$\Delta T = T_s - T_a = \frac{q_s}{h} * \varepsilon * \frac{A_{exp}}{A} = 11.5 \text{ K}$$

This means that from the internal and external temperature there is a difference of 11.5°C , which means that:

$$T_s = 40^\circ\text{C}$$

8.3.1.3 Refrigeration system design

The refrigeration system has been designed considering that the storage tank volume is completely occupied by the nitrogen (20Nm^3).

According to the scheme showed in the Figure 80, to the correct storage of EDA a flowrate $\dot{V}_p = 5 \frac{Nm^3}{h}$, able to guarantee the complete recycling of volume of tank each 4 hours, has been considered.

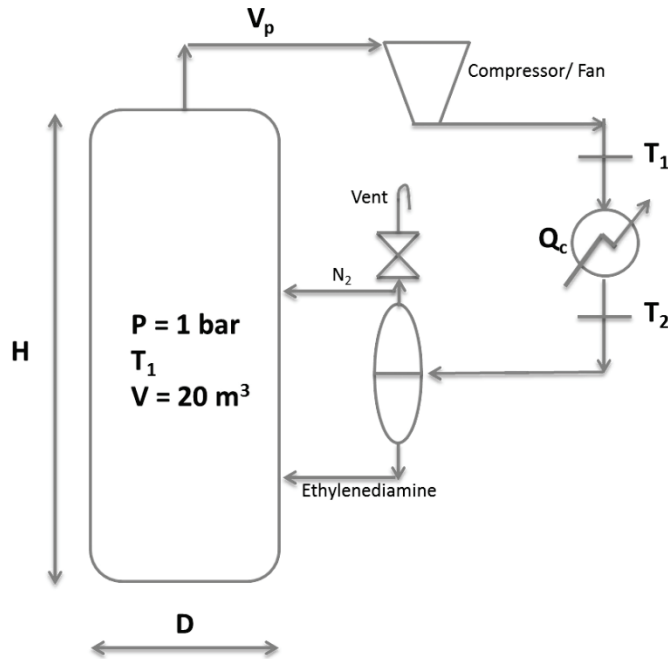


Figure 80. Storage and refrigeration scheme

Considering an internal tank temperature $T_1 = 40^\circ C$, which is the inlet temperature to the refrigeration system, and an outlet temperature from the refrigeration system $T_2 = 15^\circ C$, is possible calculate the heat exchanger power:

$$\dot{Q}_c = \rho * c_p * \dot{V} * \Delta T + \dot{m}_{EDA} * \lambda_{EDA}^{ev}$$

To determine the ethylenediamine mass in the fumes, it is necessary analyze the liquid-vapor equilibrium existing and for this the Raoult's law has been considered:

$$y_{EDA} * P = x_{EDA} * P_{EDA}^{SAT}$$

Considering that the liquid phase is completely composed by ethylenediamine, $x_{EDA} = 1$, and considering that the vapor pressure of EDA at the T_1 is $P_{EDA}^{SAT} = 0.0532 \text{ bar}$, the EDA gas molar fraction is:

$$y_{EDA} = 0.0532$$

By virtue of this, the nitrogen molar fraction is:

$$y_{N_2} = 0.9468$$

To determine the mass to treat every cycle is necessary calculate the total moles and for this scope the ideal gas law is applied:

$$P * V = n * R * T$$

Where R is the ideal gas constant: $R = 8.314 \frac{J}{mol K}$.

By the ideal gas law: $n = 192.11 mol$.

Considering the volumetric flowrate, the molar flowrate is:

$$\dot{n} = 192.11 \frac{mol}{h}$$

Table 56. Nitrogen and EDA properties

Properties	N ₂	EDA
ρ [kg/m ³]	1.131	0.897
c_p [kJ/kgK]	1.042	2.55
λ^{ev} [kJ/kg]		628.02
PM [g/mol]	28	60.1

Considering the molar fraction of EDA is possible calculate the mass of ethylenediamine treated every cycle:

$$\dot{m}_{EDA} = y_{EDA} * PM_{EDA} * \dot{n} = 0.614 \frac{kg}{h}$$

Considering a molecular weight of mixture $PM_{mix} = 29.71 \frac{g}{mol}$, it is possible to calculate the mixture mass flowrate:

$$\dot{m}_p = 5.71 \frac{kg}{h}$$

To solve the thermal balance is necessary to calculate the mixture properties and for this reason is necessary to calculate previously the mass fraction:

$$Y_{EDA} = 0.108$$

$$Y_{N_2} = 0.892$$

$$\rho_{mix} = 1.106 \frac{kg}{m^3}$$

$$c_{p_{mix}} = 1.204 \frac{kJ}{kgK}$$

By virtue of this is possible to solve thermal balance:

$$\dot{Q}_c = \rho_{mix} * c_{p_{mix}} * \dot{V} * \Delta T + \dot{m}_{EDA} * \lambda_{EDA}^{ev}$$

$$\dot{Q}_c = 155 W$$

To store the ethylenediamine at 15°C, the design of storage system results in the necessity of a ventilator of $5 \frac{m^3}{h}$, an heat exchange of 155 W, a gas-liquid separator and all the necessary described equipment described.

Chapter Nine

Realization of the industrial plant

9.1 Picture of the industrial plant

In the following pictures is showed the storage tanks area with the storage tanks of ethylenediamine, organic mixture and phenol in the upper picture, and the storage tanks of sodium hydroxide, process water in the lower picture.



Figure 81. Storage tanks area

In the Figure 82 is showed the phenol storage tank thermally traced before (on the left) and after (on the right) the insulation.

From the picture is possible to observe as the piping, the valves and the instruments also are thermally traced to avoid the phenol solidification in the pipeline.



Figure 82. Phenol storage tank before (on the left) and after (on the right) insulation

In the following picture is showed the production plant of iron chelates with the drying section in the close-up and the reactor on the background.



Figure 83. Production plant

In the following picture are showed some external and internal details of reactor and in the Figure 86 is displayed the drying section.



Figure 84. External view of reactor (on the left) and internal view of reactor (on the right)



Figure 85. Detail of the top of reactor (on the left) and flow meters (on the right)



Figure 86. Drier

9.2 Scale-up and economic analysis

The scale-up of process has been realized according the parameters listed in the Table 57.

Table 57. Scale-up specifications

Productivity:	1.25 tons/day
Working hours	8 h/day
Monthly working day	20
Yearly working day	240
Energy cost	0.03 €/kWh
Work cost (2 operators)	14 €/day
Purge factor	2%

Table 58. Daily consumption and costs

	Weight [kg]	Cost [€/day]
Phenol	105	115.5
Ethylenediamine	145.8	583
Glyoxylic acid	718.5	2371.2
Sodium hydroxide	388.2	116.5
Water	7169	215
Iron sulfate (III)	705.7	211.7
Energy		187.2
Employees cost		192

The economic analysis of the scale-up has been realized considering the process described before and without considering the first working days (start-up), when big amounts of phenol and toluene are necessary to prepare the organic mixture to recycle during the productions.

Based on this analysis, the daily costs are 3992.5 € and considering the production of 1250 kg of iron chelated every day, this means a cost of 3.19 €/kg of product.

Chapter Ten

Conclusions

10.1 Conclusions

Analyzing the literature available on the production of iron chelates, it is clear as these extremely diffused products are produced according to processes developed in the second part of last century which involve the enormous use of organic solvents, chlorinated substances and water. Although these processes have been optimized, they are not environmentally sustainable and imply high costs of post processing operations and disposal of dangerous substances

Based on this scenario, it is clear the necessity to develop a production process environmentally friendly, based on the elimination of chlorinated substances and the reduction of organic solvents use.

The first aim of this research job have been reached developing a process in which, starting from the reference industrial process developed in 70's [14], the chlorinated substances have been replaced with not chlorinated compounds (synthesis #1).

Nevertheless, this has been only the first step because the most important part of work has been aimed to the reduction, as much as possible, of the use of organic solvent. By testing several reaction fed ratios, the best compromise between reaction results and reduction of solvents volume have been successfully found. After the optimization of organic solvent fed, the volumes of others solvents employed in the process have been reduced, too, with a further reduction of environment and cost impacts of the process, and a complete management of the operating parameters have been reached.

The positive results obtained have permitted to successfully test the recycling, in a closed loop, of the organic solvents used in the process with a drastic reduction of process environment impact. This ambitious goal have been reached and a new milestone of an industrial process have been placed by the development of a process more environmental sustainable, less expansive and with a reduced reaction time.

The protocols developed for the syntheses #9 and #12, with the optimization of reaction time, the reduction of solvents involved in the process and their recycling in a closed loop, and the optimization of the iron salt use, represent two applicable industrial processes which

can be further optimized from the point of view of the increasing of reaction yield, the increasing of the ortho-ortho EDDHA chelated iron amount and the reduction of costs.

Based on the developed process, an industrial plant has been designed on behalf of Fertenia SrL, the company which support this research project.

The industrial plant, according to the company requirements, has been designed to ensure the productivity $3 \cdot 10^6 \frac{kg}{year}$ of chelated iron by the industrialization of #9 and #12 syntheses protocols.

The material and energy balances have been solved and, based on the process design the batch plant has been considered able to reach the target productivity.

The design of industrial plant included the jacketed reactor, the reagents and products storage tanks, the process control system, the utilities equipment and the drying system.

The drying system has been a critical part of plant design, considering that from the reactor is obtained an iron chelated water solution of 14% w/w which has to dry at a concentration of 98% w/w, with high energy costs which have been reduced by the design of an optimized thermal integrated network..

The building of the industrial plant has been supported in every phase in order to ensure the plant realization according to the project such as to permit the correct process industrialization.

The last step of the this work, at the end of building phase, and beyond the scope of the thesis, will be the start-up of the industrial plant for the production of the chelated iron for agricultural use, by the application of the optimized synthesis protocol.

Appendix A

Material balances

Material balances

Based on the plant scheme proposed in Figure 87 and considering the process parameters listed in Table 59, the characteristics of compounds involved in the process presented in Table 60 and the compounds index of Table 61 the material balances have been solved. The results are reported in Table 62 and Table 63.

Table 59. Material balances parameters

Parameter	Value
Productivity [kg/day]	1250
Reaction yield [%]	23
o,o-EDDHA/Fe ³⁺ [%]	2.5
Organic solvents/ethylenediamine ratio	37
Phenol/ethylenediamine ratio	26
Toluene/ethylenediamine ratio	11
Water/ethylenediamine ratio during extraction phases	148
Organic solvents/ethylenediamine ratio during extraction phases	37
Separation efficacy	1
Iron/ethylenediamine ratio	0.72

Table 60. Chemical characteristics of compounds involved in the process.

Compound	Molecular weight [g/mol]	Density [g/cm ³]
Phenol	94.11	1.07
Toluene	92.14	0.87
Glyoxylic acid	74.04	1.34
Ethylenediamine	60.10	0.90
Caustic soda	40.00	1.50
Water	18.00	1.00
Iron salt	404.00	
EDDHA	360.36	
EDDHA/Fe	416.00	

N.B. As density of Caustic soda and glyoxylic acid are considered the values of aqueous solutions 50% w/w.

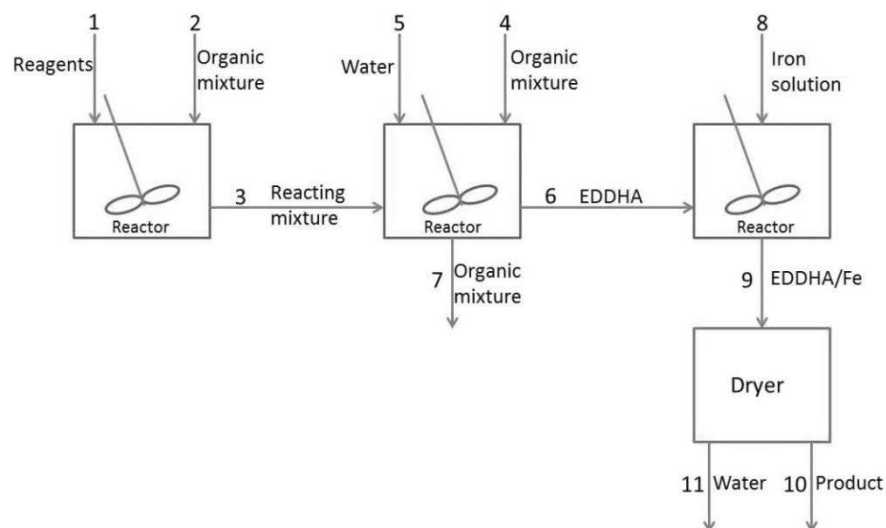


Figure 87. Plant scheme used for the material balances

Table 61. Index of substances involved in the process

Index	Compound
1	Phenol
2	Toluene
3	Glyoxylic acid
4	Ethylenediamine
5	Caustic soda
6	Water
7	Iron salt
8	EDDHA
9	EDDHA/Fe

Table 62. Results of material balances on molar basis

	n1 [kmol]	n2 [kmol]	n3 [kmol]	n4 [kmol]	n5 [kmol]	n6 [kmol]	n7 [kmol]	n8 [kmol]	n9 [kmol]	n10 [kmol]	n11 [kmol]
1	1.12	62	62	62.5	0	0	124.47	0	0	0	0
2	0	27	27	27.27	0	0	54.3	0	0	0	0
3	4.85	0	3.74	0	0	3.74	0	0	3.74	3.74	0
4	2.43	0	1.87	0	0	1.87	0	0	1.87	1.87	0
5	4.85	0	3.74	0	0	3.74	0	0	3.74	3.74	0
6	30.74	0	30.74	0	359.1	389.83	0	39.21	429.04	0	429.04
7	0	0	0	0	0	0	0	1.75	1.18	1.18	0
8	0	0	0.56	0	0	0.56	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0.56	0.56	0

Table 63. Results of material balances of weight basis

	m1 [kg]	m2 [kg]	m3 [kg]	m4 [kg]	m5 [kg]	m6 [kg]	m7 [kg]	m8 [kg]	m9 [kg]	m10 [kg]	m11 [kg]
1	105	5831.6	5831.6	5882.1	0	0	11713.7	0	0	0	0
2	0	2491	2491	2512.6	0	0	5003.6	0	0	0	0
3	359.3	0	276.6	0	0	276.6	0	0	276.6	276.6	0
4	145.8	0	112.3	0	0	112.3	0	0	112.3	112.3	0
5	194.1	0	149.5	0	0	149.5	0	0	149.5	149.5	0
6	553.4	0	553.4	0	6463.5	7016.9	0	705.7	7722.6	0	7722.6
7	0	0	0	0	0	0	0	705.7	480.3	480.3	0
8	0	0	201.1	0	0	201.1	0	0	0	0	0
9	0	0	0	0	0	0	0	0	232.1	232.1	0

Based on the results of the material balances, it has been possible to calculate the volumes of substances used during the process.

Table 64. Volumes involved in the phases of process

	V1 [m ³]	V2 [m ³]	V3 [m ³]	V4 [m ³]	V5 [m ³]	V6 [m ³]	V7 [m ³]	V8 [m ³]	V9 [m ³]	V10 [m ³]	V11 [m ³]
1	0.098	5.45	5.45	5.497	0	0	10.947	0	0	0	0
2	0	2.88	2.88	5.784	0	0	5.784	0	0	0	0
3	0.536	0	0.413	0	0	0.413	0	0	0.413	0.413	0
4	0.162	0	0.125	0	0	0.125	0	0	0.125	0.125	0
5	0.259	0	0.199	0	0	0.199	0	0	0.199	0.199	0
6	0.553	0	0.553	0	6.463	7.017	0	0.706	7.723	0	7.723
7											
8											
9											

To design the reactor, the storage tanks and all the necessary equipment, is useful a table to summarize the volumes used for each phase of process.

Table 65. Summary of volumes used in each process phases

Reaction	Volumes [m³]
Phenol	0.10
Organic mixture	8.33
Glyoxylic acid	0.41
Ethylenediamine	0.12
Caustic soda	0.20
Tot.	9.17
Extraction phases	
Water	6.46
Organic mixture	8.40
Tot.	14.87
Extracted solvents	
Organic mixture	16.73
Chelation	
Iron solution	1.05

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