Analysis of iron chelates in commercial iron fertilizers by gel chromatography

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Summary – Zusammenfassung

A gel chromatographic method for the quality control of iron chelate fertilizers is described. The iron chelates are separated on a column of Sephadex G-10 and the eluates are analysed for iron. Using a sample quantity of 25 mg in a volume of 5 ml water and eluting with 0.15 M soidum chloride solution, a separation was achieved of commercial products of Fe-EDDHA or Fe-EDDHMA.

The chromatographic analysis of Fe-EDTA or Fe-DTPA calls for a better resolution. This was obtained by decreasing the sample quantity and eluting with 0.035 M calcium chloride solution of pH 7.0. In this way it is possible to eliminate the interference of iron chelates of moderate stability which can be present in commercial products containing Fe-EDTA or Fe-DTPA.

Bestimmung von Eisen in Eisen-Handelsdüngemitteln mittels Gelchromatographie

Mit Hilfe der Gelchromatographie wurde eine Bestimmungsmethode für Eisenchelate entwickelt, welche in der landwirtschaftlichen und gartenbaulichen Praxis angewandt werden kann. Das Verfahren wird als Standardmethode bei der Qualitätsprüfung von Handelspräparaten empfohlen. Die Eisenchelate wurden auf einem Gelbett von Sephadex G-10 aufgetrennt. Nach Eluierung konnten die Eisenchelate mittels einer Eisenanalyse bestimmt werden.

Die Auftrennung von Fe-EDDHA oder Fe-EDDHMA erfolgt am besten durch Elution einer Substanzmenge von 25 mg mit einer 0.15 M NaCl-Lösung.

Für die Analyse von Fe-EDTA oder Fe-DTPA in Handelspräparaten genügen diese Bedingungen nicht. Um ihre Bestimmung quantitativ durchzuführen und Störungen durch nicht stabile Eisenchelate zu eliminieren wird eine Substanzmenge von 2 mg mit einer 0.035 M $CaCl_2$-Lösung, pH 7.0, eluiert.

Introduction

In the years that have elapsed since the beginning of the use of synthetic iron chelates as a means of correcting iron deficiency in plants, little attention has been given to the development of analyses for the quality control of commercial iron chelates. In addition to the content of water-soluble iron, it is also customary to determine the content of chelated iron in the products.

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However, the content of chelated iron does not guarantee that all the chelated iron in the products is present in the form of Fe-EDTA, Fe-DTPA or Fe-EDDHA. The effectiveness of an iron chelate does not only depend on its content of chelated iron, but also on the stability of the chelated iron in the soil in the presence of competing ions. This stability is determined by the nature of the chelating agent. If the chelated iron compound contains impurities in the form of iron chelates of moderate stability, its value as an iron fertilizer will be less. Therefore it is desirable to develop a uniform method of analysis for the various chelates that are available on the market. The method should be based on a separation technique, which must be specific for anyone of the constituent iron chelates. Various chromatographic methods, as paper and thin layer chromatography, ion-exchange and gas chromatography (*Hill-Cottingham*, 1962, *Rajabalee* et al. 1973, *Longbottom*, 1972, *Aue* et al. 1972) have been used in this context. Almost all suffer from cation interference, lack of sensitivity or inability to determine more than one or two chelating species. This paper describes a gel chromatographic analysis suited for the determination of individual iron chelates and meeting the criteria described.

Materials and methods

Materials

A number of commercial products as well as analytically pure iron chelates were tested. The following compounds were involved in this study: Fe-EDTA (ethylenediamine tetraacetic acid); Fe-DTPA (diethylenetriamine pentaacetic acid); Fe-EDDHA (ethylenediamine di (o-hydroxyphenylacetic acid) and Fe-EDDHMA (ethylenediamine di (2-hydroxy-4-methylphenylacetic acid)). The pure iron chelates were obtained by mixing equimolar quantities of $FeCl_3 \cdot 6H_2O$ and the sodium form of EDTA, DTPA and EDDHA. The compounds used were analytically pure (Merck; Light).

Sample solution

Iron chelate solutions were made by shaking 0.5 g of the compounds with 100 ml deionized water. The solutions were filtered and stored in the dark.

Eluents

All reagents were of reagent grade.

Eluent 1: 0.15 M sodium chloride solution, adjusted to the pH of the iron chelate solutions with hydrochloric acid or sodium hydroxide.
Eluent 2: 0.035 M calcium chloride solution, adjusted to pH 7.0 with sodium hydroxide.

Preparation of Sephadex columns

Sephadex G-10 and G-50 medium, which are cross-linked dextran gels, were used as the bed material.

The dry Sephadex powders were suspended in the eluent and allowed to settle for four hours. A slurry of the prepared gel was poured into the column (l = 34 cm; diam. = 2.6 cm). After settling, the column was washed with the eluent for two hours at a rate of 2 ml/min.

Procedure

A sample application cup was inserted in the top of the column. A 2- or 5-ml volume of the sample solution was brought into the cup just as the last drops of the eluent soaked into the bed. When the last portion of the sample solution passed into the bed the eluent was reapplied. The effluent was collected in fractions of 5 ml. The amount of solute in the fractions was determined as follows. In the case of Fe-EDTA and Fe-DTPA the column effluent was monitored at 260 nm with a spectrophotometer (*Hill-Cottingham,* 1957). The effluent of Fe-EDDHA and Fe-EDDHMA was measured at 485 nm. Also fractions containing an iron chelate were compounded and analysed after digestion with Fleischmann acid for total iron with a modified phenanthroline method (*Van Driel,* 1964). The iron content of this composite fraction was expressed as a percentage of the total soluble iron content of the chelated iron compound.

Results and discussion

Fe-EDDHA and Fe-EDDHMA

Fig. 1 shows the elution pattern of pure Fe-EDDHA and two commercial Fe-EDDHA products. As eluent was used 0.15 M NaCl solution, which was adjusted to the pH of the iron chelate solutions.

Fig. 1 shows, that the elution pattern of product A is very similar to the pattern of pure Fe-EDDHA. However, the elution pattern of product B is quite different. Besides a strong peak of a brown coloured compound, the elution curve only shows a weak peak of Fe-EDDHA. Fig. 2 demonstrates the elution curves of two Fe-EDDHMA products with the same eluent. Since pure Fe-EDDHMA could not be obtained, these products could not be compared which pure Fe-EDDHMA. On account of the colour and the elution volume of the fractions it may be concluded that the third peak represents Fe-EDDHMA.

Replicates of the gel chromatograms show that the location and the width of the peaks are identical, provided that the conditions as the length of the column, eluent and chelate concentration remain unaltered. As all fractions contain iron, it is clear that products B, C and D are contaminated with other iron compounds. From figs 1 and 2 it can be seen which fractions must be pooled for the determination of Fe-EDDHA and Fe-EDDHMA. In these fractions iron was determined and from the values found and the total soluble iron contents it is possible to calculate percentages of iron present as Fe-EDDHA or Fe-EDDHMA in the products (Table 1).

The results indicate that the products B, C and D are of a low grade.

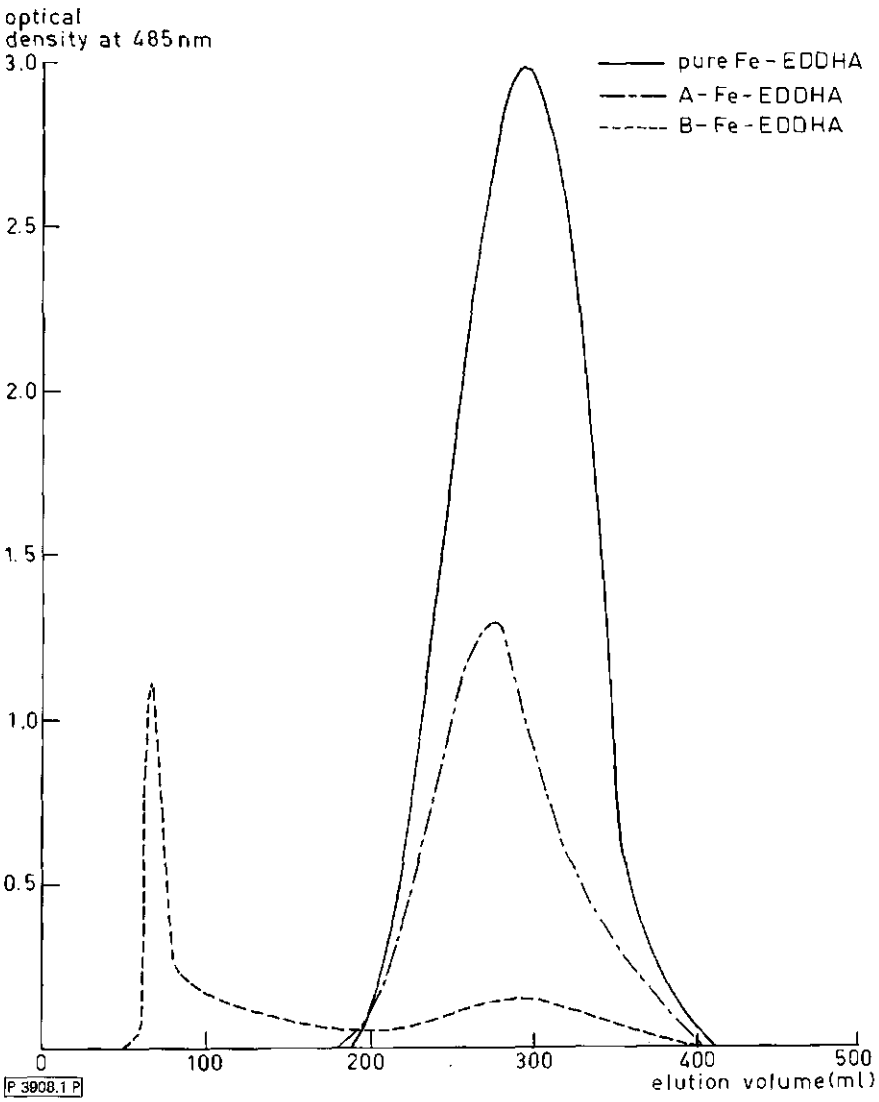

Figure 1: Elution curves of Fe-EDDHA samples on Sephadex G-10. Eluent: 0.15 M NaCl solution. Sample quantity: 25 mg

Abbildung 1: Elutionskurven von Fe-EDDHA Produkten an Sephadex G-10. Elutionsmittel: 0.15 M NaCl-Lösung. Substanzmenge: 25 mg

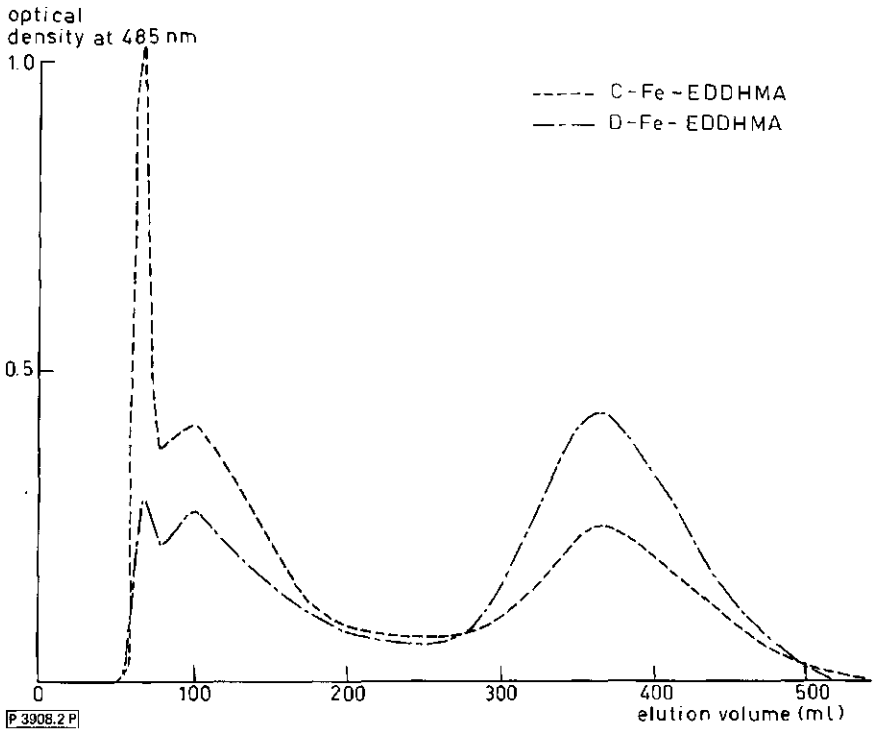

Figure 2: Elution curves of Fe-EDDHMA samples on Sephadex G-10. Eluent: 0.15 M NaCl solution. Sample quantity: 25 mg
Abbildung 2: Elutionskurven von Fe-EDDHMA Produkten an Sephadex G-10. Elutionsmittel: 0.15 M NaCl-Lösung. Substanzmenge: 25 mg

Table 1: Soluble iron contents of Fe-EDDHA and Fe-EDDHMA products before and after gel chromatography
Tabelle 1: Gehalte an wasserlöslichem Eisen in Fe-EDDHA und Fe-EDDHMA Produkten vor und nach Gelchromatographie

Description	Total soluble iron content %	Soluble iron content of the iron chelates after gel chromatography %	Purity of the iron chelates, expressed as a percentage of the total iron content %
Pure Fe-EDDHA	12.75	12.72	99.8
A – Fe-EDDHA	6.20	5.82	93.9
B – Fe-EDDHA	5.35	1.63	30.5
C – Fe-EDDHMA	5.84	2.90	49.7
D – Fe-EDDHMa	4.55	2.87	63.1

Fe-EDTA

Fig. 3 gives the elution pattern of pure Fe-EDTA and two Fe-EDTA products under the same conditions as in fig. 1 and 2. Their elution patterns show a good resemblance, but the peaks are asymmetrical with skewed leading edges. This phenomenon enlarges the width of the peak and results in a decrease of the resolution capacity. In the collected fractions that contain Fe-EDTA, again iron was determined and expressed in the same way as in Table 1. From the data in Table 2 it can be seen that the soluble iron in the products E and F is mainly present as Fe-EDTA.

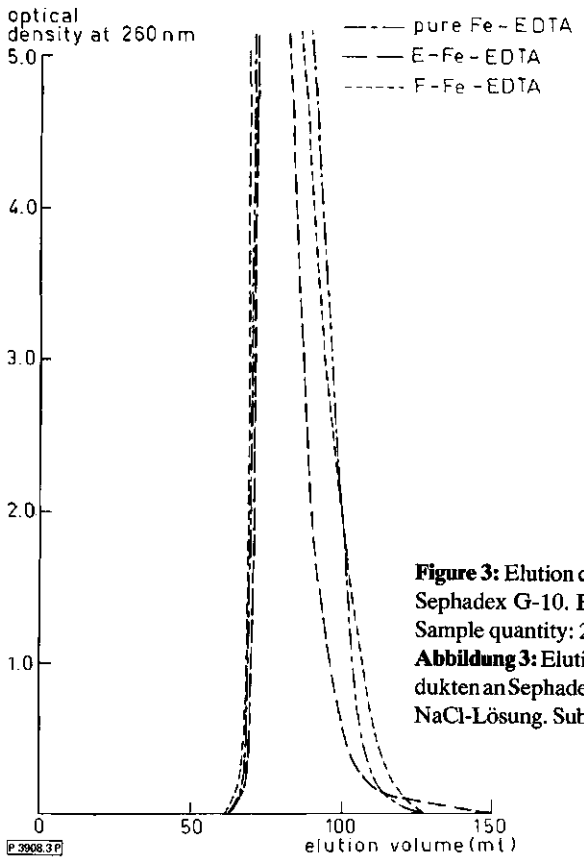

Figure 3: Elution curves of Fe-EDTA samples on Sephadex G-10. Eluent: 0.15 M NaCl solution. Sample quantity: 25 mg
Abbildung 3: Elutionskurven von Fe-EDTA Produkten an Sephadex G-10. Elutionsmittel: 0.15 M NaCl-Lösung. Substanzmenge: 25 mg

In order to investigate the interference by iron chelates of moderate stability, gel chromatograms were made for Fe-NTA (nitrilotriacetic acid), Fe-EDTA and a mixture of Fe-NTA and Fe-EDTA (Fig. 4). There was an overlap of Fe-EDTA and Fe-NTA, making it impossible to separate a mixture of Fe-EDTA and Fe-NTA.

Table 2: Soluble iron contents of Fe-EDTA products before and after gel chromatography
Tabelle 2: Gehalte an wasserlöslichem Eisen in Fe-EDTA Produkten vor und nach Gelchromatographie

Description	Total soluble iron content	Soluble iron content of the iron chelates after gel chromatography	Purity of the iron chelates, expressed as a percentage of the total soluble iron content
	%	%	%
Pure Fe-EDTA	13.40	13.32	99.4
E – Fe-EDTA	4.81	4.73	98.3
F – Fe-EDTA	13.32	13.11	98.4

To eliminate the interference due to iron chelates of moderate stability, a 0.035 M calcium chloride solution, adjusted to pH 7.0, was used as eluent. Because of the competing effect of calcium ions at pH 7.0, partial displacement of iron from chelates with a low stability will take place. This effect has been observed when Fe-NTA is eluted on the column with the calcium chloride solution of pH 7.0. A great part of its iron is retained on the column (Table 3).

Table 3: Soluble iron in five samples of iron chelates, before and after gel chromatrography, using sample sizes of 2 mg and calcium chloride solution as eluent
Tabelle 3: Gehalte an wasserlöslichem Eisen in fünf Proben von Eisenchelaten vor und nach Gelchromatographie bei Verwendung von 2 mg Substanzmenge und Elution mit $CaCl_2$ Lösung

Description	Total soluble iron	Recovery of iron from Fe-EDTA after gel chromatography	Recovery of iron from Fe-NTA after gel chromatography
	µg/2 mg sample	µg/2 mg sample	µg/2 mg sample
Pure Fe-EDTA	267.6	266.9	
Mixture of 1 mg Fe-EDTA and 1 mg Fe-NTA	358.1	142.6	
Pure Fe-NTA	448.9		86.2
E – Fe-EDTA	96,5	89,6	
F – Fe-EDTA	265,4	254,7	

Also the effect of the amount of iron chelate on the shape and the width of the elution peaks of Fe-EDTA and Fe-NTA was investigated. Fig. 5 shows elution curves of different amounts of Fe-EDTA, eluted with calcium chloride solution. It appears that the elution volume of the Fe-EDTA peak decreases with decreasing sample size. Moreover, at the smallest amount of Fe-EDTA the elution peak is almost symmetrical. The same results have been observed for Fe-NTA. Since at all

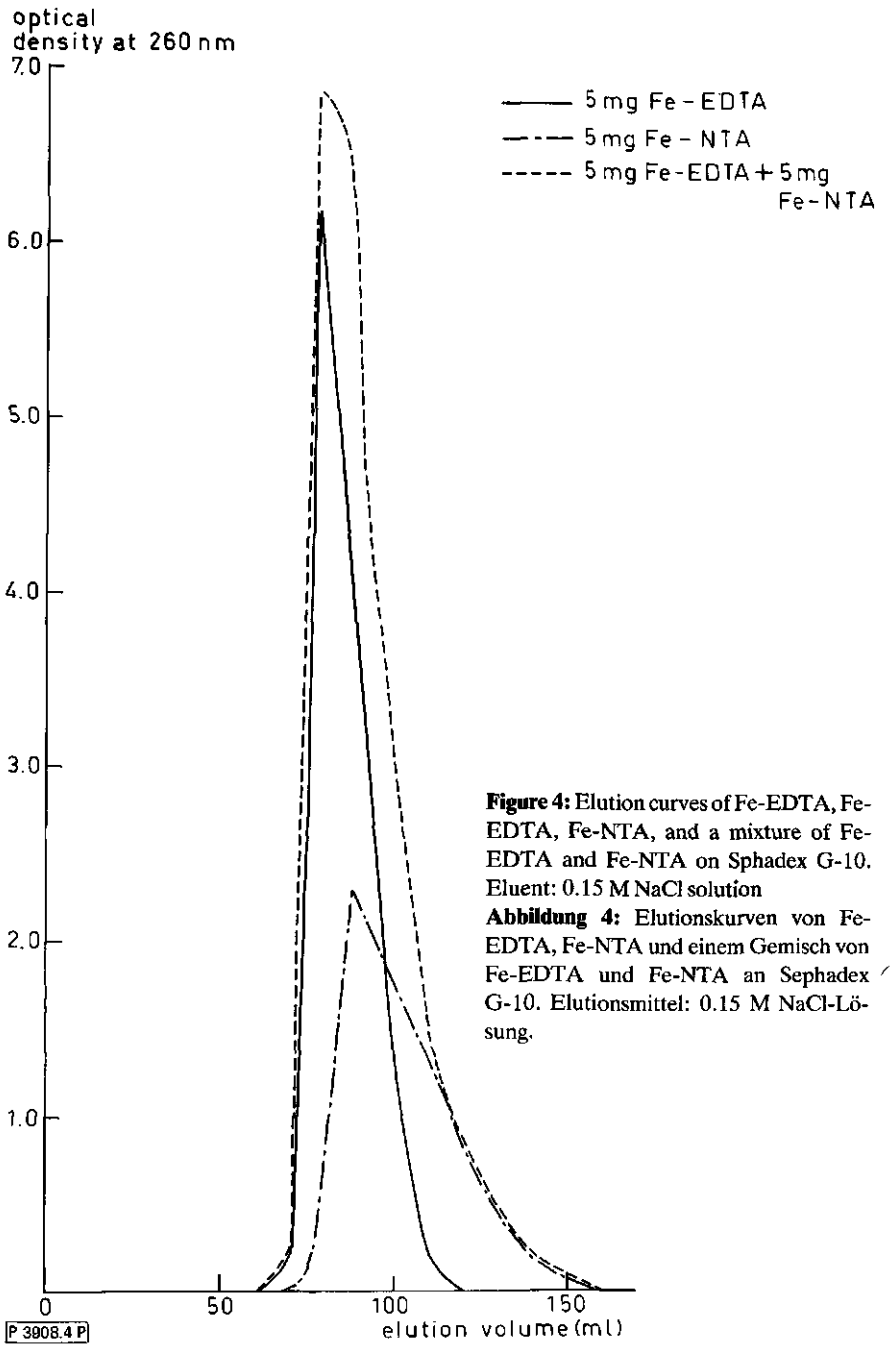

Figure 4: Elution curves of Fe-EDTA, Fe-EDTA, Fe-NTA, and a mixture of Fe-EDTA and Fe-NTA on Sphadex G-10. Eluent: 0.15 M NaCl solution

Abbildung 4: Elutionskurven von Fe-EDTA, Fe-NTA und einem Gemisch von Fe-EDTA und Fe-NTA an Sephadex G-10. Elutionsmittel: 0.15 M NaCl-Lösung.

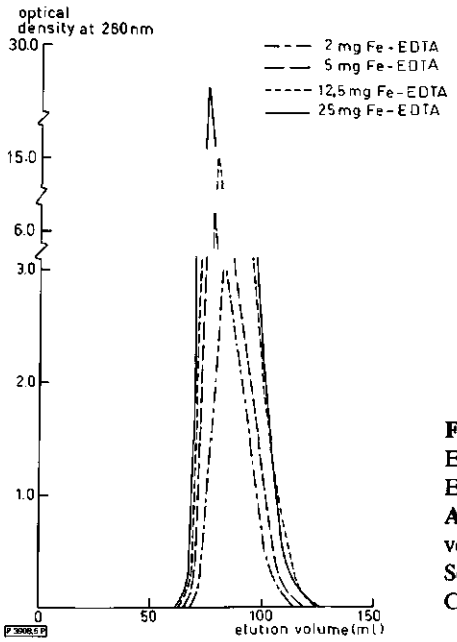

Figure 5: Dependence of elution curves on Fe-EDTA sample quantity on Sephadex G-10. Eluent: 0.035 M $CaCl_7$ solution (pH 7.0)
Abbildung 5: Abhängigkeit der Elutionskurven von der Fe-EDTA Substanzmenge an Sephadex G-10. Elutionsmittel: 0.035 M $CaCl_2$-Lösung (pH 7.0)

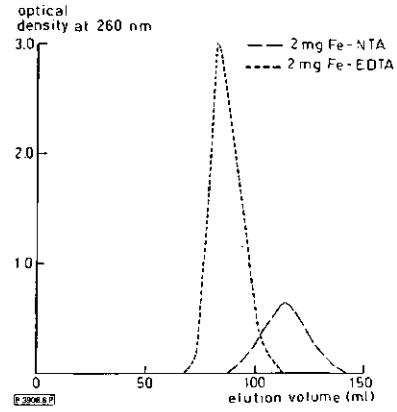

Figure 6: Elution curves of Fe-EDTA and Fe-NTA on Sephadex G-10. Eluent: 0.035 $CaCl_2$ solution (pH 7.0). Sample quantity: 2 mg
Abbildung 6: Elutionskurven von Fe-EDTA and Fe-NTA an Sephadex G-10. Elutionsmittel: 0.035 M $CaCl_2$-Lösung (pH 7.0). Substanzmenge: 2 mg

sample quantities of Fe-NTA a great part of the iron was retained on the column, their elution curves only represent a part of the amounts of Fe-NTA.

Fig. 6 illustrates that only a slight overlap of Fe-EDTA and Fe-NTA occurs when sample quantities of 2 mg are used.

Iron analyses in the pooled fractions of Fe-EDTA, Fe-NTA and a mixture of Fe-EDTA and Fe-NTA indicated that, using sample quantities of 2 mg, the

interference of Fe-NTA in the analysis of Fe-EDTA is small (Table 3). Also it is seen that about 80 % of the iron from Fe-NTA is retained on the column. If the products E and F were analyzed for Fe-EDTA (Table 3), using a sample size of 2 mg and calcium chloride solution as eluent, somewhat lower percentage of purity were found than in Table 2.

The reproducibility of the method was measured for six replicates of sample quantities of 2 mg of pure Fe-EDTA. The analyses yielded a mean value of 267.7 µg Fe ± 2.1 with a relative standard deviation of 0.8 %.

Fe-DTPA

The elution patterns of pure Fe-DTPA and two Fe-DTPA products were practically identical, if a sample quantity of 25 mg and sodium chloride solution as eluent were applied. On the other hand, the shapes of the peaks were asymmetrical, while especially the peak of product H shows a straight skewed edge (Fig. 7). By

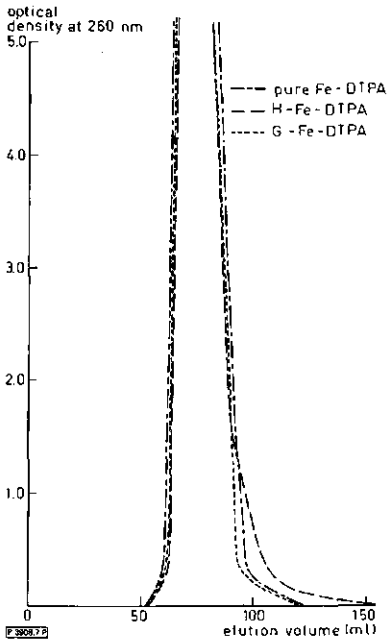

Figure 7: Elution curves of Fe-DTPA samples on Sephadex G-10. Eluent: 0.15 M NaCl solution. Sample quantity: 25 mg
Abbildung 7: Elutionskurven von Fe-DTPA Produkten an Sephadex G-10. Elutionsmittel: 0.15 M NaCl-Lösung. Substanzmenge: 25 mg

using calcium chloride solution as eluent and a sample quantity of 2 mg, the shape of the peaks becomes more symmetrical and a smaller elution volume of the peaks is obtained (Fig. 8). Moreover, the elution curve of product H shows a second, very small peak, which must be attributed to an impurity of product H. After collecting the Fe-DTPA fractions according to both methods, iron was determined again in the

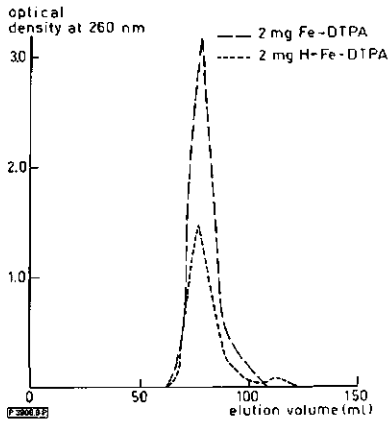

Figure 8: Elution curves of Fe-DTPA samples on Sephadex G-10. Eluent: 0.035 M $CaCl_2$-solution (pH 7.0). Sample quantity: 2 mg
Abbildung 8: Elutionskurven von Fe-DTPA Produkten an Sephadex G-10. Elutionsmittel: 0.035 M $CaCl_2$-Lösung (pH 7.0). Substanzmenge: 2 mg

Table 4: Soluble iron contents of Fe-DTPA products before and after gel chromatography
Tabelle 4: Gehalte an wasserlöslichem Eisen in Fe-DTPA Produkten vor und nach Gelchromatographie

Description	Total soluble iron content	25 mg sample, eluent: NaCl solution		2 mg sample, eluent: $CaCl_2$ solution	
		Soluble iron content of the iron chelates after gel chromatography	Purity of the iron chelates expressed as a percentage of the total iron content	Soluble iron content of the iron chelates after gel chromatography	Purity of the iron chelates expressed as a percentage of the total iron content
	%	%	%	%	%
Pure Fe-DTPA	11.81	11.81	100.0	11.80	99.9
G – Fe-DTPA	9.82	9.82	100.0	9.73	99.1
H – Fe-DTPA	7.01	6.60	94.2	6.17	88.0

fractions (Table 4). A sample quantity of 2 mg, eluted with calcium chloride solution, gives for product H a lower percentage of purity than a sample quantity of 25 mg, eluted with sodium chloride solution.

Even when sample quantities of 2 mg and elution with calcium chloride solutions are used, the elution peaks of Fe-DTPA and Fe-EDTA overlap. To improve the resolution, a gel with a higher porosity was tried. With Sephadex G-50 the retention volumes of Fe-DTPA and Fe-EDTA increased as can be expected in view of the molecular sieve effect, but no better separation of the chelates was obtained. If the product contain a mixture of Fe-EDTA and Fe-DTPA, its total chelated iron content can be determined by the gel chromatographic procedure, but it is not possible to determine the content of each iron chelate in this way. However, the method

described offers excellent possibilities for quality control of commercial products of single iron chelates. The examination of the products only involves a determination of the total soluble iron and an iron analysis in the pooled fraction after the gel chromatographic separation. The contribution of iron chelates of low stability is eliminated by this method.

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