Liquid chromatographic determination of ethylenediaminetetraacctic acid as metal complexes on a porous graphitic carbon column

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Abstract

A novel liquid chromatographic system for determination of ethylenediaminetetraacetic acid (EDTA), using a complex-forming metal ion [Fe(III)] in the eluent was developed. Optimisation of the system was obtained after a careful choice of complex-forming metal and pH of the eluent. The porous graphitic carbon column permits use of a wide pH range and due to its high hydrophobicity it is possible to determine EDTA without addition of ion-pair reagents to the eluent. The chromatographic behaviour of the Fe(III) complex, the free EDTA and Fe(III) ions were considered and possible pitfalls due to equilibrium disturbances are shown and discussed. Applications to analysis of EDTA in local anaesthetic parental solutions and determination of nitrilotriacetic acid as an impurity in EDTA substance are given.

1. Introduction

Ethylenediaminetetraacetic acid (EDTA) is a strong complex-forming agent widely used as a metal-masking additive in e.g. the pharmaceutical and the food industries. EDTA forms complexes with several metals and is suitable as an additive where metal ions may cause problems like e.g. metal-catalysed degradation and occurrence of coloured metal complexes. In the pharmaceutical industry there is a need for determination of EDTA both as a quality control test on the substance itself and as an assay of EDTA in different formulations.

EDTA has been determined with different techniques, e.g. titrimetric analysis [1,2], gas chromatography [3], UV spectrophotometry [4], flame atomic absorption spectrometry [5], isotachophoresis [6] and electroanalytical methods [7]. During the last decade several ion chromatographic and reversed-phase liquid chromatographic methods were presented [8–21].

In the LC methods the content of EDTA is often determined as metal complexes. In some cases occurrence of "ghost peaks" and peak splitting is reported [9,11,12,19]. The cause of these effects has been briefly discussed, but seems to be due to equilibrium disturbances.

This study is an effort to describe and explain the causes of such disturbances, in order to optimise the chromatographic system. A method was developed using a system involving one

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dominating metal complex. To avoid disturbances the metal ion was added to the eluent.

The method described has been applied to the determination of EDTA and nitrilotriacetic acid (NTA) in pharmaceutical samples.

2. Experimental

2.1. Reagents and chemicals

EDTA and NTA were obtained as disodium salts, Titriplex III and Titriplex I, from E. Merck, Darmstadt, Germany. A Carbocaine adrenaline formulation containing EDTA was obtained from Astra Pain Control, Södertälje, Sweden. All other chemicals were of analytical-reagent quality or better.

2.2. Chromatographic system

The liquid chromatographic system consisted of parts obtained from Spectra-Physics (San José, CA, USA). The pump was a Model 8800. The autosampler, 8880, was modified with a Valco CV6H injector. The UV detector was a Spectra 200. A Chrom-Jet integrator connected to a Chromstation computing system was used to collect and interpret data. The column was a Hypercarb 100×4.6 mm I.D., $10-\mu$m particles from Shandon (Cheshire, UK).

2.3. Procedures

The eluents used were aqueous solutions containing 2% ethylene glycol. Different buffering additives were used, at pH 1.5 a sulphuric acid buffer and at pH 5.0 an acetate buffer. The eluents containing metal ions were obtained by adding iron(III) sulphate or copper(II) acetate. The sample solutions were prepared by adding a 2–3-fold molar excess of the metal salt compared to EDTA. All solutions were prepared from doubly distilled water.

The injected sample volume was 20 μl. The flow-rate was set at 1.0 ml/min and detection was by UV. EDTA as acid was detected at 220 and 240 nm, the copper complex at 254 nm and

the iron complex at 270 nm, due to differences in absorption maxima for free EDTA and the two complexes.

3. Results and discussion

3.1. EDTA

EDTA is a multidentate agent which is able to form very strong complexes with various metal ions [22]. The complex-forming ability of EDTA increases with increasing pH since the $EDTA^{4-}$ ion forms the complex [22]. However, at high pH, undissolved metal hydroxides may decrease the complex formation. The influence of pH on the complex formation for several metal ions is shown in Fig. 1 (cf. Ref. [22]). The conditional complex-forming constant is plotted against the pH. As shown, e.g. at pH 7 the conditional constant of Cu(II) is higher then that of Fe(III), about 2 log units. Thus Cu(II) is preferably chosen as complex-forming metal ion in this pH region. However, interference from Ni(II) may occur (cf. Fig. 1). At pH 5 the ratio of the conditional constant of Fe(III) and Cu(II) is reversed. If Cu(II) is chosen as complexing
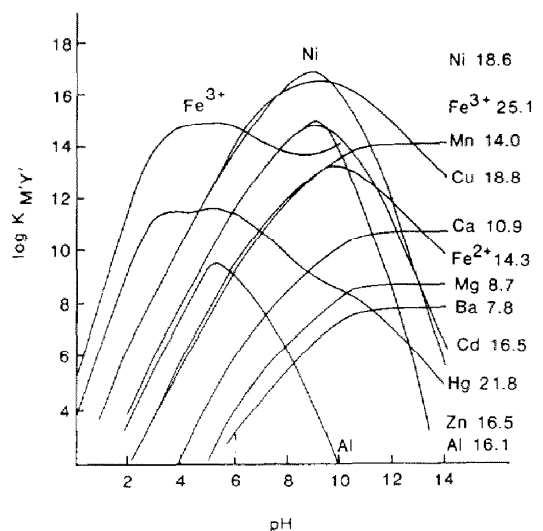

Fig. 1. Complex-binding constants of EDTA with various metal ions: influence of pH. From Ref. [22]. Stability constants are given in the margin.

metal ion strong interference will appear in the presence of Fe(III). Also Ni(II), Hg(II), Cd(II) and Zn(II) may cause interference.

The ability of Fe(III) to form strong complexes at low pH can be used to increase the selectivity. Ringbom [1] showed that Fe(III) can be used in quantitative titrimetric determination of EDTA at pH 1. In this pH region other metal ions show low ability to form complexes with EDTA (Fig. 1).

3.2. Chromatographic system

In reversed-phase liquid chromatography, EDTA is mainly determined as metal complexes in systems where a counter-ion is added to the mobile phase [11–17,20,21]. The retention of the EDTA complexes will depend on the charge and type of metal complex, the surface concentration of the counter-ion, the ionic strength and the dielectric constant of the mobile phase [23].

In other systems EDTA itself is used as a complex-forming agent in the eluent to achieve separation between metal ions [10,24,25].

In the present study the main purpose was to interpret the complex-forming behaviour of EDTA in the chromatographic system. To simplify the LC system the counter ion was not included. This will remove potential disturbances which may occur if the EDTA peak is co-eluted in the counter-ion system peak [26,27].

To obtain a suitable retention of EDTA without addition of a ion-pair reagent, the column surface must be strongly hydrophobic. Furthermore, it would be desirable if the column is stable within a wide pH range.

The Hypercarb column (porous graphitic carbon) possesses the qualities mentioned. EDTA is retained in a buffer-containing eluent, but a low percentage of an organic additive, ethylene glycol, was added to stabilise the detection signal. Ethylene glycol has only a minor effect on the retention of EDTA.

3.3. Choice of complex-forming metal ion

In the literature the copper ion was used as complexing ion in some papers [8,9,11,12,15,19]

and peak distortion was reported in some of them [9,11,12,19].

The Cu(II) ion is suitable for complex binding to EDTA in the pH range 7.5–12. At lower pH interference from other metal ions e.g. the Fe(III) ion will be pronounced and effects of such interferences have been observed in LC systems [11,19]. The choice of pH is crucial. Even though it has been known for some decades that the complex-forming ability of EDTA is strongly pH dependent, unfavourable pH conditions were used in several papers [8,9,11,15,19].

To study the effects of unfavourable conditions for complex formation in the LC system used in the present investigation, the Cu(II)–EDTA complex was chosen. Cu(II)–EDTA was injected in a LC system containing acetate buffer pH 5.0 with 1 mM Cu(II) ions in the eluent. An additional peak eluted before the Cu(II)–EDTA peak (Fig. 2A) and by adding Fe(III) ions to the sample, the peak increased significantly and we suggest its identity to be the Fe(III)–ETDA complex (Fig. 2B).

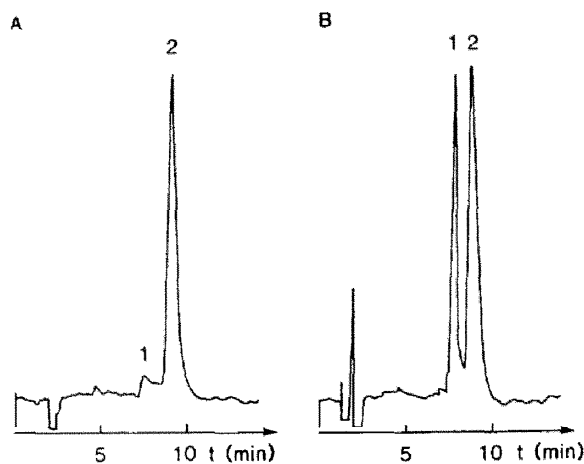In spite of the fact that Cu(II) ions were

Fig. 2. Interference of Fe(III) ions in a system with an eluent containing Cu(II) ions at non-optimal conditions. Solutes: (A) EDTA 0.27 mM in mobile phase; (B) EDTA 0.27 mM + iron(III) sulphate 0.07 mM. Wavelength: 254 nm. Mobile phase: acetate buffer 0.05 M, pH 5.0 with 1 mM Cu(II) and 2% ethylene glycol. Peaks: (A) 1 = unknown, 2 = Cu(II)–EDTA; (B) 1 = Fe(III)–EDTA, 2 = Cu(II)–EDTA.

present in the eluent, they were exchanged for Fe(III) ions in the EDTA complex. This is due to the fact that the complex-forming constant of Fe(III) ions is higher compared to the Cu(II) ions, the logarithmic values of the conditional complex-forming constants are 14.9 and 12.3, respectively, at pH 5.0.

Fe(III) ions are always present as an impurity in the eluent in LC systems with stainless-steel parts, which was confirmed by an atomic absorption determination of Fe(III) ions.

The eluent contained a Fe(III) concentration of 90 μM.

Restrictions in choice of complex-forming metal will appear due to the high Fe(III) concentration in the background and the Fe(III) ions were chosen as complex-forming ions in further studies.

Fe(III) ions form a stable complex with EDTA at low pH values and a high selectivity in complex formation is obtained under these conditions (cf. Fig. 1).

The stability of the Fe(III) complex in the described LC system was further investigated by adding Ni, Cu, Cr and Zn ions to a Fe(III)–EDTA sample. No "ghost peaks" or peak distortions were obtained in the LC system with a sulphuric acid buffer pH 1.5 and 0.1 mM Fe(III) ions added to the eluent.

3.4. Chromatography of EDTA and Fe(III)–EDTA

Injection of the acid form of EDTA in a LC system with no metal ions added to the eluent, resulted in an EDTA peak with an anomalous band spreading (Fig. 3A).

The band spreading can be explained by secondary equilibria causing different distribution to the stationary phase. These secondary equilibria are most likely due to trace amounts of metal ions from the chemicals applied and the metal parts in the LC system. The band spreading can be suppressed either by removal of interfering metal ions or by addition of a metal which forms a strong complex with EDTA.

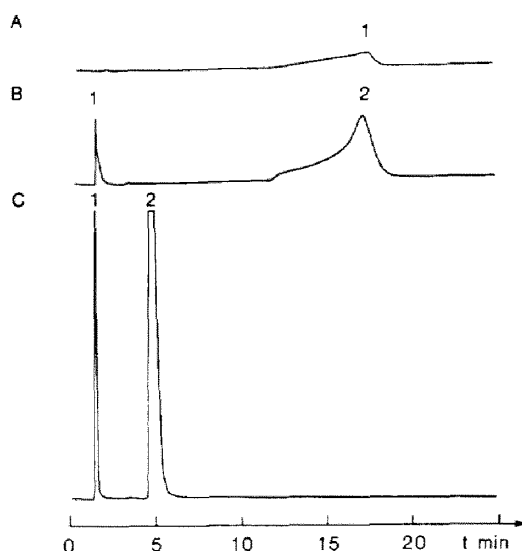When a sample of Fe(III)–EDTA is injected

Fig. 3. Effect of addition of Fe(III) ions to the mobile phase. Solutes: (A) EDTA 2.70 mM; (B, C) EDTA 2.70 mM + 5.90 mM iron(III) sulphate. Wavelengths: (A, B) 220 nm; (C) 270 nm. Mobile phases: (A, B) sulphuric acid buffer 0.1 M, pH 1.5, 2% ethylene glycol; (C) as in A and B but 0.1 mM iron(III) sulphate is added. Peaks: (A) 1 = band of EDTA; (B) 1 = iron(III) sulphate, 2 = anomalous band of dissociated Fe(III)–EDTA; (C) 1 = iron(III) ions, 2 = Fe(III)–EDTA.

into the system without addition of metal ions to the mobile phase, also a broad and anomalous band is obtained (Fig. 3B).

The result in Fig. 3B clearly shows that the complex between Fe(III) and EDTA dissociate during the elution, since no distinct peak is eluted at the retention time of the complex (cf. Fig. 3C). This is due to the fact that the retention of the free acid is much higher than the retention of the complex and that the Fe(III) ions are eluted close to the front. A similar observation was made when the complex of naproxen and albumin was chromatographed [28]. To avoid the disturbances described above the metal ion is added to the eluent.

A retention model for metal complexes in similar LC systems was proposed by Horváth et al. [29]. The retention was found to be affected by both the retention of the solute molecule, the

retention of the complex, the retention of the complex-forming agent as well as the size of the complex-forming constant,

$$k = \frac{k_0 + k_c K[H]}{1 + K[H]} \qquad (1)$$

where k is the capacity factor (k_c being the capacity factor of the complex and k_0 that of the non-complexed solute), K is the complex-forming constant and $[H]$ is the concentration of the complex-forming metal in the mobile phase.

Since the complex formation is a reversible equilibrium it is important to have an excess of the complex-forming agent in both the sample solution and in the chromatographic eluent.

From Eq. 1 derived by Horváth et al. it can be concluded that the retention of the complex is dependent on the concentration of the complexing agent in the eluent in a hyperbolic fashion. The retention is affected in a narrow concentration range in a order of $+/- 2$ log units in magnitude, where the concentration of the complex agent is $1/K$.

The complex-binding constants of EDTA with different metal ions are high, e.g. at pH 1.5 log K for Fe(III) is about 10, i.e. the retention of EDTA will be effected in the concentration range of 10 nM–1 pM for Fe(III). These concentrations are much lower compared to the normal sample concentration in LC. The fact is that the trace levels of Fe(III) from the chemicals applied and metal parts in the LC system are higher.

The conclusion is that the metal concentration will not affect the retention but the peak shapes.

Addition of Fe(III) to the eluent results in one sharp peak of Fe(III)–EDTA (Fig. 3C). The response of EDTA is also increased compared to Fig. 3A and B due to the increased molar absorptivity of the Fe(III)–EDTA complex compared to free EDTA. The concentration of Fe(III) ions in the eluent should be chosen sufficiently high to suppress the dissociation of the complex. However, the choice of the concentration in the eluent is limited, due to background absorbance. Our investigations showed that addition of 0.1 mM Fe(III) to the eluent

resulted in an acceptable absorbance background level (0.21 AU) and a excellent peak performance.

4. Applications

4.1. Determination of EDTA in a local anaesthetic solution

Carbocaine adrenaline is a local anaesthetic formulation containing several components. EDTA is added to control the influence of unwanted contamination of trace metals which may promote degradation. Inman et al. [14] managed to determine EDTA in a complex pharmaceutical mixture with a gradient ion-pair RPLC system.

The methodology described in this paper was applied and a chromatogram of a diluted sample (1:1) from the Carbocaine adrenaline solution showed high selectivity and no interferences appeared (Fig. 4). An excellent linearity was obtained in the concentration range 0.01–0.21 mg/ml ($r^2 = 0.9999$). The quantification was per-
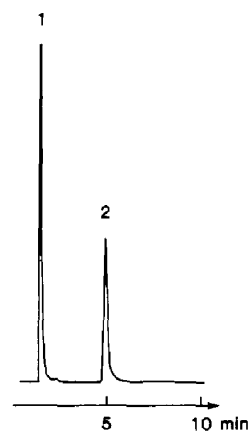

Fig. 4. EDTA in a local anaesthetic solution. Solute: Carbocaine adrenaline 20 mg/ml containing 0.25 mg/ml EDTA and a molar excess of iron(III) sulphate. Wavelength: 270 nm. Mobile phase: sulphuric acid buffer 0.1 M, pH 1.5, containing 0.1 mM iron(III) sulphate and 2% ethylene glycol. Peaks: 1 = iron(III) ions; 2 = Fe(III)–EDTA.

formed with a single-point calibration. Determination of an added amount of 0.250 mg/ml EDTA in a Carbocaine adrenaline solution resulted in a recovery of 0.248 mg/ml with a relative standard deviation of 0.8% ($n = 6$). The limit of detection was about 1 μM (0.4 μg/ml), which is similar compared to methods described in the literature [14].

4.2. Determination of NTA

NTA is determined as an impurity in EDTA, described in the US Pharmacopeia [30]. The specification limit for NTA in USP-grade disodium EDTA is maximum 0.1% (w/w). The US Pharmacopeial method developed by Parkes et al. [12] is an ion-pair RPLC method where NTA and EDTA form complexes with copper. The quantification of NTA is performed with a 10.0 mg/ml solution of EDTA and the content of NTA is determined with a standard-addition method. Determination of NTA complexes with Fe(III) in the system described in this paper is possible due to a UV response of the same order as Fe(III)–EDTA [16] and a sufficiently high complex-forming constant for NTA and Fe(III).

Fig. 5 shows a typical chromatogram. The detection limit of NTA is 0.4 μg/ml and when a 1.0 mg/ml EDTA sample was injected a detection limit of 0.05% (w/w) was easily obtained.
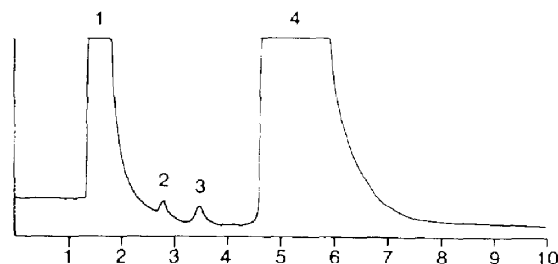

Fig. 5. Determination of NTA in EDTA. Solute: EDTA 2.70 mM (1 mg/ml) and a 3-fold molar excess of iron(III) sulphate. Wavelength: 270 nm. Mobile phase: sulphuric acid buffer 0.1 M, pH 1.5, containing 0.1 mM iron(III) sulphate and 2% ethylene glycol. Peaks: 1 = iron(III) ions; 2 = unknown; 3 = NTA; 4 = EDTA.

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