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Technical paper

Determination of Free Ethylenediaminetetraacetic Acid in Foods by Titration Using Eriochrome Black T with Visual End-Point Indication

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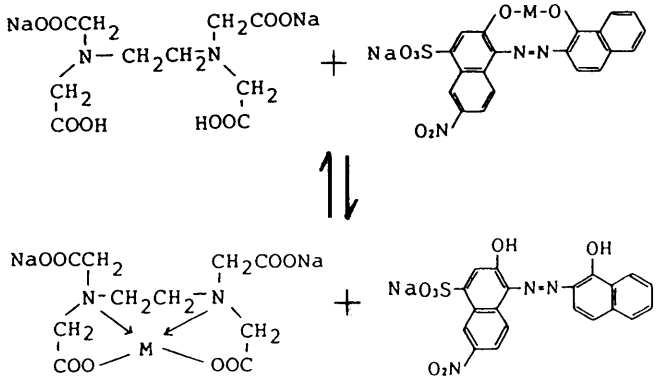
A direct titrimetric method for the determination of ethylenediaminetetraacetic acid (free EDTA) in foods is described. Free EDTA was extracted from food samples by equilibrium dialysis using ammonium buffer. An aliquot of dialyzate was subjected to direct titration with standard $MgCl_2$ solution at pH 8.5 using Eriochrome Black T as a metallochromic indicator. The common inorganic ions (PO_4^{3-}, NO_3-, Cl—) and organic acids (citric acid, tartaric acid) in foods do not interfere with the proposed methods.

Keywords: free ethylenediaminetetraacetic-acid, food, visual titration, eriochrome black T

Ethylenediaminetetraacetic acid (EDTA), as its disodium or calcium disodium salts, is widely used in foods such as mayonnaise, salad dressings and canned foods. Under Japanese regulations, the presence of EDTA and its sodium salt (referred to as free EDTA) in final products is explicitly prohibited. Therefore, it is necessary to determine whether free EDTA exists in foods.

Methods hitherto published for the determination of EDTA involve spectrophotometric (Hamano *et al.*, 1993), gas chromatographic (Mihara *et al.*, 1970), and high-performance liquid chromatographic procedures (Perfetti & Warner, 1979; Yabe *et al.*, 1982). However, any of these methods lend themselves to the determination of total EDTA (the sum of free EDTA and its metal chelates) only.

In the literature (Alvarez *et al.*, 1988), some metal ions have been determined by titration with EDTA-2Na using Eriochrome black T as the metallochromic indicator. The reaction mechanism can be written as follows:



where M represents di- or tri-valent cations. In the present study, we took advantage of the above reaction mechanism for the determination of free EDTA in foods.

Materials and Methods

Apparatus Cellulose tubing, 30 m in length, 27 mm i.d., was purchased from Wako Pure Chemicals (Osaka, Japan) and used after cutting in 150 mm lengths. The relative molecular mass cut-off for molecules of this tubing is 12000–14000 Daltons (instruction manual). The closures were also obtained from Wako for use in sealing the cellulose tubing. An Atomic absorption spectrophotometer (Hitachi Model 163) was used for measuring the metal ions.

Reagents All reagents, except the indicator, were of analytical reagent grade. The indicator, Eriochrome black T (EBT), was obtained from Dojindo Laboratories (Kumamoto, Japan). De-ionized, distilled water was used for all solutions.

Standard magnesium chloride solution, 1 mM. A 0.952 g sample of magnesium chloride, dried at 130°C for 3 h, was dissolved in 1000 ml of water. This solution was diluted to 100 ml with water.

Ammonium buffer solution (pH 8.5), 0.1 M. A 5.35 g sample of ammonium chloride was dissolved in about 500 ml of water and the pH adjusted to 8.5 with ammonium hydroxide solution, then diluted to 1000 ml with water.

Ammonium buffer solution (pH 8.5), 0.02 M. A 200 ml aliquot of 0.1 M ammonium buffer solution was diluted to 1000 ml with water.

Indicator solution, 1 mM. A 46.1 mg sample of EBT was dissolved in 100 ml of methanol. The solution was freshly prepared every month and kept in a refrigerator.

Procedure A 25 g portion of the sample was homogenized with about 60 ml of ammonium buffer solution (0.1 M, pH 8.5) and the volume was adjusted to 100 ml with distilled water. A 20 ml sample of the homogenate was introduced into the cellulose tubing of which one end had been sealed with a closure. After the other end of the tubing was similarly sealed, it was subjected to equilibrium dialysis against 180 ml of ammonium buffer solution (0.02 M, pH 8.5). This opera-

tion was carried out overnight at room temperature with occasional shaking by hand. A 40 ml aliquot of the dialyzate (corresponding to 1 g of sample) was taken in a 100 ml tall-form beaker, followed by 10 ml of ammonium buffer solution (0.1 M) and 0.1 ml of indicator solution. After the pH was adjusted to 8.5 with 10% HCl or 10% ammonium hydoxide solution, the mixture was titrated with standard magnesium chloride solution to a faint purple end-point with continuous stirring by hand.

Results and Discussion

Choice of indicator Many direct and indirect complexometric methods for the individual determination of metal ions using different metallochromic indicators have been reported (Halliday & Leonald, 1987; Hafez *et al.*, 1991). As the aim of this work is to determine free EDTA in foods, of which common di- or tri-valent metal ions are Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{3+}, conditions must be found where the indicator can be reacted with these metal ions, and the complex formed is easily decomposed in the presence of free EDTA. Of the indicators examined, EBT was found to be the most suitable for this purpose.

Effect of pH In the literature (Halliday & Leonard, 1987), it is recommended that the complexometric determination of Mg^{2+} using EBT as an indicator should be done at pH 10.0. In order to find the optimum pH conditions for the assay of free EDTA, the pH of the standard solutions containing free EDTA was varied from 8.0 to 11.0 using 10% ammonium hydroxide solution or 10% HCl. Table 1 shows the results obtained using blank, standard (EDTA-2Na) solutions. With an increase in pH, the required volume of titrant for the blank decreased. However, when the pH of the solution was raised to 11.0, the end-point could not be observed. Alvarez *et al.* (1988) stated that Mg^{2+} was precipitated as $Mg(OH)_2$ at a pH>10.5. However, in this study, the precipitate of the titrant ($MgCl_2$) did not appear even at pH 11.0, as the required volume of the titrant for the assay of free EDTA was almost zero (see Table 1). Whether the EDTA metal chelate gave the positive result or not is also a critical factor for our purpose. The EDTA-Fe chelate gave a positive error at pH values higher than 9.0, as shown in Table 1. This may be due to the slow dissociation of this chelate under strong alkaline conditions, thus leading to the release of Fe^{3+} ion. Fortunately, at pH values below 9.0, EDTA-Fe did not affect the assay of free EDTA (see Table 1). The other examined EDTA metal chelates (Ca, Mg, Sn, Zn, Pb, Cu) did not affect the titration of free EDTA at pH 8–10 (data not shown), as these chelates were relatively stable under the examined alkaline conditions (pH 8–10). Even if NH^{4+} ion can be exchanged for Na^+ or H^+ ion of EDTA-2Na under our assay conditions (pH 8.5), this phenomenon was not a serious problem for our purpose because the experimental results for the standard solution coincided with the theoretical values (see Table 1). Accordingly, titration at pH 8.5 is recommended.

Effect of amount of EBT indicator The amount of EBT was varied from 0.01 μmol to 10 μmol. At low amounts of EBT, as is shown in Table 2, the end-point could not be easily detected by the human eye, thus the end-point was beyond the expected volume of the titrant. At higher amounts of EBT, the color intensity of EBT itself was too strong and the reproducibility of the end-point became worse. Accordingly, the use of 0.2 ml of 1 mM (corresponding to 0.2 μmol) of EBT is recommended.

Selection of metal ion as titrant EBT is well known to form a metallocomplex with divalent cations such as Mg^{2+}, Ca^{2+}, Cu^{2+} and Pb^{2+} under moderate alkaline conditions (pH 8–10) (Halliday & Leonald, 1987). Therefore, these divalent cations were examined as a titrant for the assay of free EDTA. The color change with Mg^{2+} is easier to detect and more precise than that of the other metal ions examined (data not shown). Accordingly, Mg^{2+} is selected as the titrant.

Interference of foreign species The study of potential interferents was divided into three parts. The amounts of the interferents examined were limited to their possible ranges expected in commercial foods (Amakawa *et al.*, 1988; Tsuji *et al.*, 1993, 1994; Science and Technology Agency, 1982). In the first part, common inorganic anions, which form a weak complex with Mg^{2+}, were investigated. This study was carried out in the following manner. To a 40 ml sample of standard free EDTA solutions (0.1 μmol/ml), a 50-fold excess of the possible interferents (PO_4^{3-}, NO_3-, Cl−) was independently added, then the change in volume of the titrant relative to a standard containing only EDTA was determined. All the compounds tested did not interfere in the proposed method. Secondly, divalent metal ions, capable of forming a complex with EDTA, were assessed for their effect on the determination of free EDTA. If the amounts of divalent cations such as Ca^{2+} and Mg^{2+} were in excess of that of free EDTA in food

Table 1. Effect of pH on the end-points of the titration of free EDTA.

pH	Titrant (ml)		
	Blank	Standard	Standard+ EDTA-Fe
8.0	0.75	1.26 (0.51)[a]	1.26 (0.51)
8.5	0.15	0.65 (0.50)	0.65 (0.50)
9.0	0.10	0.60 (0.50)	0.45 (0.35)
10.0	0.07	0.57 (0.50)	0.09 (0.02)
10.5	0.05	0.56 (0.51)	0.08 (0.03)
11.0	0	0 (0)	0 (0)

Forty ml of standard (free EDTA: 0.5 μmol) or mixture of standard and EDTA-Fe (0.5 μmol) was titrated with $MgCl_2$ (1 mM) solution at indicated pH. Theoretical net volume of titrant for standard is 0.5 ml. [a] Data in parentheses represent net volume of titrant. The pH of the solutions, basically composed of ammonium buffer solution (pH 8.5), were adjusted to the indicated value with 10% HCl or 10% ammonium hydroxide solution.

Table 2. Effect of amount of EBT indicator on the titration of free EDTA.

Amount of EBT (μmol)	Titrant (ml)[a]		
	Blank	Standard	Net
0.01	0.05 (1.2)[b]	0.65 (1.1)	0.60
0.05	0.05 (1.1)	0.60 (1.1)	0.55
0.1	0.1 (1.2)	0.60 (1.2)	0.50
0.2	0.15 (1.5)	0.55 (1.7)	0.50
1.0	0.22 (1.7)	0.71 (1.5)	0.49
5.0	0.27 (2.5)	0.79 (2.7)	0.52
10.0	0.43 (3.0)	0.95 (3.4)	0.52

Forty ml of standard (free EDTA: 0.5 μmol) was titrated with $MgCl_2$ (1 mM) solution at pH 8.5 using a different amount of indicator. Theoretical net volume of titrant for standard is 0.5 ml. [a] Mean values of three trials. [b] Data in parentheses represent the relative standard deviation.

Table 3. Recovery of free EDTA from several foods.

Food[a]	Added (μmol/g)	Found (μmol/g)	Recovery (%)
Mayonnaise	20	9.6	48.0 (98.9)[b]
Canned sardine	50	26.8	53.6 (97.1)
Canned crab meat	50	14.0	28.0 (95.9)
Canned mushroom	20	12.5	62.5 (97.6)

[a] Contents of divalent metal ions (the sum of Ca^{2+} and Mg^{2+}) in mayonnaise, canned sardine, canned crab meat and canned mushroom were 10.3, 22.5, 35.4 and 7.2 μmol/g, respectively. [b] Data in parentheses were calculated by subtracting the amounts of EDTA-metal chelate with Ca^{2+} and Mg^{2+} from the amounts of EDTA-2Na added.

samples, no free EDTA may exist in the sample, due to the formation of a complex with those metal ions. This means that there is no need to analyze free EDTA for such food samples.

In the third part, some organic compounds commonly present in commercial foods were tested for their possible interference with the proposed method. Citric and tartaric acids were not significant interferent at a concentration of 1%.

Removal of protein When the assay of free EDTA was directly applied to real samples such as mayonnaise, rich in proteins, lipids and/or emulsifying agents, a change in color could not be observed (data not shown). This can be ascribed to the complex formation of the indicator (EBT) with protein (Alvarez *et al.*, 1988). However, the use of equilibrium dialysis at the extraction step eliminates their interference. Thus, an important feature of the present method is that extraction of free EDTA is performed using equilibrium dialysis, which allows some interferents such as proteins and emulsifying agents as well as lipids to be removed.

Recovery Recovery studies were performed on several commercial foods, which had been verified to be free from EDTA. Considering the contents of divalent metal ions in the foods examined, the spiked level of EDTA-2Na was set above the level of the divalent metal ions based on molar ratio. Known amounts of EDTA-2Na were added to the sample prior to the initial homogenization step, then analyzed according to the method described in the Material and Method section. The results are given in Table 3. As is obvious from Table 3, the experimental values for recovery were 48–62% of the added values. The reason why the experimental values are lower than those expected from the added level of EDTA-2Na is that some part of the recovered EDTA was in a chelate form with Ca^{2+} and/or Mg^{2+} ions due to an equimolar reaction. The contents of other divalent metal ions in foods tested can be negligible (data not shown). When the experimental values were corrected for EDTA-metal chelate, they (more than 95%) became close to the expected value.

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