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Determination of EDTA in Dairy Wastewater and Adjacent Surface Water

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***Abstract*—**An HPLC-UV analytical method was developed to determine ethylenediaminetetraacetic acid (EDTA) in dairy wastewater and surface water. The optimizing separation was achieved by reversed–phase ion-pair liquid chromatography on a C18 column using methanol as mobile phase solvent, tetrabutylammonium bromide as the ion-pair reagent in pH 3.3 formate buffer solution at a flow rate of 0.9 mL min^{-1} with a UV detector at 265 nm. No interference of Ca, Mg or NO_3^- was detected. Method performance was evaluated in terms of linearity, repeatability and reproducibility. The method detection limit was 5 µg L^{-1}. The contents of EDTA in dairy effluents were 72 ~ 261 µg L^{-1} at a large dairy site. A change of EDTA concentration was observed downstream of the dairy effluent discharge, but this was well under the predicted no effect concentration for aquatic ecosystem.

***Keywords*—**Dairy wastewater, EDTA, HPLC, surface water.

I. Introduction

CLEANING of equipment for production plants is a key process in the dairy industry to ensure safe products with high quality. It has been demonstrated [1] that using synergic effects of single components in chemical mixtures, for instance adding an effective complexing agent, is more likely to achieve an improved cleansing result with lower concentration compared to aqueous solutions merely containing the basic component of sodium hydroxide. The main function of complexing agents in a cleaning solution is to prevent precipitation of calcium, magnesium and heavy metal salts, which can cause deposits to appear on both the cleaning equipment and the plant to be cleaned [2].

At present, ethylenediaminetetraacetic acid (EDTA) is the cheapest and most suitable complexing compound for many technical purposes, and is used in large quantities [3]. In the New Zealand dairy industry, EDTA has been used as a cleaning additive to improve the cleansing efficiency during the clean–in–place (CIP) procedure.

Nearly all applications eventually result in the release of EDTA to the aquatic environment [2], [3]. In the late 1980s, the environmental impacts of EDTA were scrutinized in Europe, as EDTA occurred at a higher concentration in surface waters than any other identified anthropogenic organic compounds that may cause further environmental issues [3]-[5]. In New Zealand, dairy effluent is commonly discharged into the adjacent surface water. However, there is concern about large volumes of wastewater with a high EDTA content discharged into relatively small rivers [3], [4].

The commonly used methodology for EDTA determination in samples of different matrices is based upon complexing with iron (III) as an analyte that is the most stable complex compound (pKa = 25), employing UV as a detecting method [6]-[12].

In the present study, a method using an isocratic HPLC system, a C18 reversed-phase (RP) column and UV detector has been developed. The chromatographic separation was optimized by compositions of the mobile phase and flow rate. The interfering compounds of calcium/magnesium and nitrates at levels occurring in dairy waste waters were investigated, and the accuracy and precision of the method were determined. The applicability of this method was demonstrated by analyzing dairy wastewater and surface water samples. Analyzed results demonstrated levels of EDTA in dairy effluents from a large dairy site. Contents of EDTA in the adjacent river showed the change of EDTA concentrations due to the dairy effluent discharge.

II. Experimental

Apparatus

The HPLC system consisted of a Shimadzu LC – 10 AT VP Liquid Chromatography (USA) with a 50 µL sample loop, a Shimadzu SPD – 10A VP UV - Vis detector set at 265 nm, a Hypersil C_{18} RP column (Phenomenex) of length 200 mm, diameter 4.6 mm and particle size 5µm, and a Phenomenex security guard column. The HPLC recording and integration software was PowerChrom (eDAQ Pty Ltd, Australia) attached to a Powerlab/8sp data recorder (ADInstrument). All water was obtained from an ELGAS TAT ® UHQII system and filtered through 0.45µm Nylon filters (Phenomenex). Degassing of the mobile phase was achieved by passing helium sparging.

Reagents, Chemicals and Solutions

Reagents were all chromatographic analysis grade or reagent grade used without further purification. A sodium formate / formic acid buffer solution (pH 3.3) was prepared by dissolving 0.17g sodium formate (BDH) and 0.33 ml (Ajax Finchem) 90% formic acid in 1 L of water. An ion-pair reagent solution (15 mM TBABr) was prepared by dissolving 4.836g of tetra-n-butylammoniumbromide ($C_{16}H_{36}NBr$, 322.38 g mol^{-1})

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(Merck) in 1L of pH = 3.3 buffer solution. A stock EDTA standard solution (0.1 g L^{-1} EDTA) was prepared by dissolving 0.1462 g ethylenediaminetetraacetic acid iron sodium salt (MW = 421.10 g mol^{-1}) (Merck) in 1L of water, and stored in the refrigerator wrapped in tin foil. Standard solutions, ranging from 0–750 µg L^{-1} EDTA for calibration, were prepared daily from the stock solution. A Fe^{3+} solution (0.1941g L^{-1} or 3.47 mM) was prepared by dissolving 2.4203 g $FeCl_3 \cdot 6H_2O$ (Merck) and 0.144mL HCl (37% Merck) in 500 mL water. A nitrate solution (1 g L^{-1}) was prepared by dissolving 0.4077 g of KNO3 (Seelze–Hannover) in 250 mL water as a stock solution for further dilution. Calcium (0.1 g L^{-1}) and magnesium (0.1 g L^{-1}) ion solutions were prepared by dissolving 0.2732g of CaCl2· 6H2O (BDH) and 0.1046 g of $MgCl_2 \cdot 6H_2O$ (BDH) in 500 mL water respectively for further dilution.

Sample Collection

Dairy wastewaters were 24–hour composite flow-proportional samples including plant processing wastewater, wastewater treatment processing sample and dairy effluents, which were collected at varied sites in August, October and December, 2007.

Surface water samples were collected at 2500 m (site 1) and 10 m (site 2) upstream and 10 m (site 3) and 60 m (site 4) downstream of dairy effluent discharges in the adjacent river in August, October, 2007. Sampling sites are shown in Fig. 1. The river was approximately 6-8 m wide and 1-2 m deep. Surface water samples included a morning and an afternoon sample which were combined by two different samples from the morning and afternoon, respectively.

All samples were collected in opaque PE bottles to avoid photolysis of the Fe(III)EDTA and refrigerated at 4^0C till analyzing.

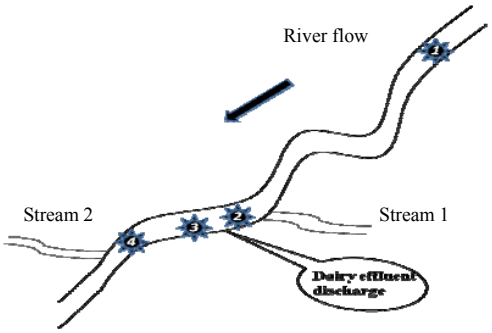

Fig 1 Sampling sites of surface waters upstream and downstream of the dairy effluent discharge

Sample Pre-Treatment

Sample pre-treatment of dairy effluents involved taking 1–5 mL aliquots, adding appropriate Fe^{3+} solution to the test tube depending the predicted EDTA concentration, leaving overnight in the dark to allow complexing of Fe(III)EDTA, filtering through 0.45 µm cellulose nitrate filters (Phenomenex), and injecting 50 µL sample into the HPLC system at ambient temperature.

Sample pre-treatment of surface water involved heating 10 mL water sample to dryness at 90 ^{0}C oven, adding 1.5 mL mobile phase and 0.5 mL 1.94 mg L^{-1} Fe^{3+} solution, leaving overnight in dark to complex, filtering and injecting 50 µL of sample into the HPLC system. This gave a five-fold pre-concentration of the EDTA before analyses.

III. Results and Discussion

A. Optimizing Chromatographic Separations

A number of HPLC methods have been published to determinate EDTA in multi-media samples [6]-[12]. A review of the literature indicated that the method of Loyaux-Lawniczak et al. [7] seemed the most appropriate. This method was published as suitable for natural waters.

The aim of HPLC separations in our case was to ensure that the analytical component of $[Fe(III)EDTA]^-$ completely separated from other compounds in dairy wastewater samples, with a practical separation time of less than 10 minutes, and to ensure that other metal – EDTA complexes were totally converted into $[Fe(III)EDTA]^-$ before analyses. The method was thus optimized for a dairy wastewater matrix, including checking possible interferences at levels found in dairy waste waters. A different pre-treatment was also found to be needed.

In selecting a particular buffer, the buffer capacity and its UV absorbance must be taken into account. Buffer capacity is determined by pH, buffer pKa and buffer concentration. The buffer range of k-formate / formic acid is 2.8 – 4.8 and the UV cutoff is 210 nm (10 mM) (absorbance < 0.5) [13]. The pH value of 3.3 was chosen as 99.2% of Fe(III)EDTA exists in its deprotonated form [7]. Furthermore, there is no absorbance at the wavelength of 265 nm [13].

In reversed phase (RP) separations, the sample retention can be controlled by varying the solvent strength of a mobile phase. This can be achieved either by using different solvents or varying the percent organic (% B) composition with the same solvent in the mobile phase. Both acetonitrile (ACN) [6], [8] and methanol (MeOH) [7], [10], [12] were investigated as solvents. A similar retention time was achieved using a lower percentage of ACN (1%) than MeOH (5%) if other parameters remained the same. The study of different % B compositions of MeOH showed that increasing % MeOH shortened the retention time. Buffer solution with 2% MeOH was selected for giving a practical retention time and a good separation ($2<k'<10$). Methanol, rather than ACN was chosen because of its low toxicity and cost.

An addition of the ion–pair reagent to the mobile phase can often improve peak shapes and large changes in separation selectivity for ionic samples [13]. The ion–pair reagent, tetrabutylammonium (TBA) bromide (TBABr) [7], [8], [10] / TBA hydroxide [6], [9], [11] / TBA hydrogen sulfate [10], [12], is often used as TBA^+ is positively charged on its nitrogen and completes with anions, for instance, $Fe(III)EDTA^-$, NO_3^-, Cl^- to form an ion-pair. The varied concentrations of TBABr in the mobile phase were studied, and the results observed that the retention of Fe(III)EDTA compound decreased when the concentration of TBABr was increased and other parameters remained the same, with a 100 µg L^{-1} EDTA standard solution (Fig. 2). The concentration of 15mM TBABr was selected for the determination of EDTA in the dairy waste water.

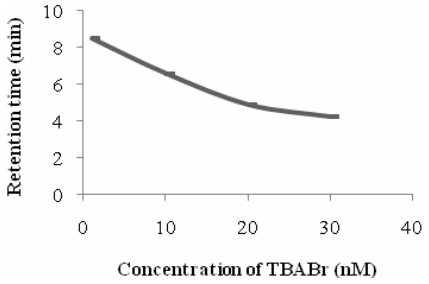

Fig. 2 Effect of concentration of TBABr on retention of Fe(III)EDTA

Retention time increases with a lower flow rate, but the separation often improves (Fig. 3). The flow rate was set at 0.9 mL min^{-1} for a better separation with a practical analysis time.

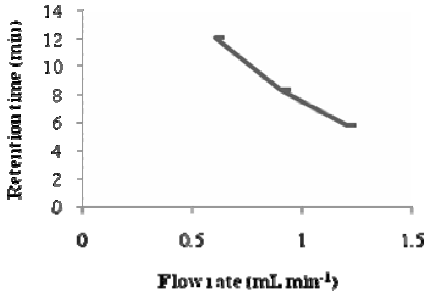

Fig. 3 Effect of flow rate of mobile phase on retention of Fe(III)EDTA

The HPLC separation for Fe(III)EDTA of interest in the dairy waste water is shown in Fig. 4 by varying solvent strengths and ion-pair reagent TBABr concentrations with the optimized flow rate (0.9 mL min^{-1}). The optimal solvent strength and concentration of TBABr were 2% methanol and 15mM TBABr for a best resolution, respectively.

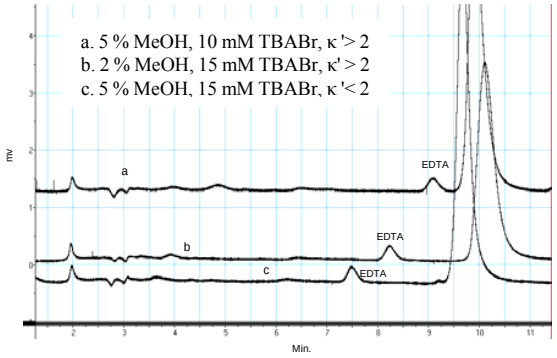

Fig. 4 Opitimazation of chromatographic separation of dairy wastewaers

B. Interfering Compounds

In general, waste waters from dairy processing plants contains high concentrations of organic material and inorganic compounds such as NO_3^-, Ca^{2+} and Mg^{2+} with large variations in pH. Organic substances do not generally interfere with the determination of EDTA. However, the determination of EDTA could be under-estimated in the presence of a high concentration of NO_3^- [7], [11].

The concentration of nitrate in dairy waste waters varies depending upon the process. Levels of nitrate found in dairy wastewater were below 100 mg L^{-1}. An experiment was undertaken by adding different concentrations of NO_3^- (10, 50 and 100 mg L^{-1}) to a 100 µg L^{-1} EDTA standard solution. There seemed to be no interference to the Fe(III) EDTA peak.

Nowack et al. [8] stated that waters with high calcium and magnesium ions may influence the determination of EDTA due to the matrix effect. The content of calcium in dairy effluent was significantly increased comparing with local clean water (8 ~ 10 times) at a large dairy site studied. To investigate this interference, an experiment was carried out by spiking a 100µg L^{-1} EDTA standard solution with different concentrations (10, 25, 50 and 100 mg L^{-1}) of Ca^{2+} and Mg^{2+} respectively. The effect of the mixture of Ca^{2+} and Mg^{2+} (4:1) was also studied. The overall results showed no significant interference of these metals on the HPLC determination of EDTA.

In dairy waste waters, EDTA exists mainly in the form of CaEDTA and MgEDTA. These species have low pKa value and slow exchange kinetics [8]. Pre-treatment is thus needed to convert these species to Fe(III)EDTA for the analysis of total EDTA in a sample. A series of experiments were carried out which involved adding different levels (C_{EDTA}: C_{Fe3+}=1:0.194) of excess Fe^{3+} (1x Fe^{3+}, 1.5 x Fe^{3+}, 2 x Fe^{3+}, 5 x Fe^{3+}, 10 x Fe^{3+} and 20 x Fe^{3+}) to a 100 µg L^{-1} EDTA standard solution under different pre-treatment conditions. The procedure involved:

- Heating in 90°C water bath for over 3 hours [7], [8];
- Placing in a dark place over night [12]; and
- Boiling for 1.5 hours.

The experimental results revealed that (i) the addition of excess Fe^{3+} levels appeared not to affect the determination of EDTA (peak areas) except by disturbing the baseline and shifting the peak retention time of the chromatogram at higher concentration of iron (Fig. 5); and (ii) similar results were obtained with different pre-treatment conditions. Consequently, the overnight pre-treatment was applied for subsequent experiments.

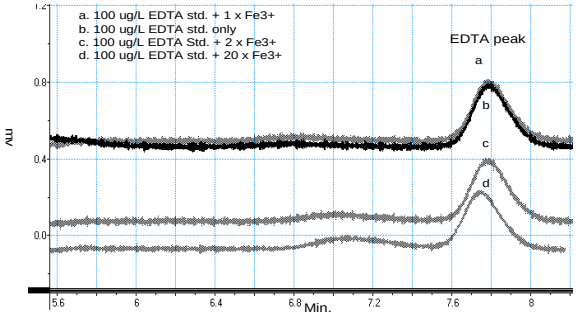

Fig. 5 Overlay of chromatograms by adding different levels of iron to a 100 µg L^{-1} EDTA standard solution. Conditions: Column, Hypersil C_{18} RP column (Phenomenex) of length 200 mm, diameter 4.6 mm and particle size 5 µm; Mobile phase, 2% MeOH, 15 mM TBABr in pH 3.3 sodium formate/formic acid buffer solution; sample loop, 50 µL; Flow rate, 0.9 mL min^{-1}; Wavelength, 265 nm

C. Method Accuracy, Precision and Detection Limit

There is no Certificated Reference Material (CRM) available for EDTA in waters. The method accuracy was checked using the analyte spike recovery, which 100 µg L^{-1} of EDTA was added dairy wastewater samples. The spike recoveries were between 98 % and 102 % (n = 9). Calibration curve (concentration versus peak area) was obtained with EDTA concentrations ranging from 0 to 750 µg L^{-1} (0, 10, 50, 100, 200, 500 and 750 µg L^{-1}) of a standard solution for dairy wastewater, which were prepared daily from the stock solution of 0.1 g L^{-1} of EDTA. Good linearity and reproducibility were observed during the experiment. Precision of the method was determined by analyzing each sample 12 times (Table I). The repeatability given as the relative standard deviation was less than 1.5 %. The method detection limit (MDL) was calculated on three times the standard deviation of sample 1 (3*1.43 µg/L), giving 5 µg/L EDTA.

TABLE I
METHOD REPEATABILITY TEST RESULTS FROM REPLICATE DAIRY WASTEWATER ANALYSES

Tests	Sample 1 (µgL^{-1})	Sample 2 (µgL^{-1})
1	107.2	1792.4
2	107.9	1759.3
3	107.1	1758.9
4	107.7	1780.3
5	107.5	1756.3
6	106.9	1737.5
7	106.2	1801.1
8	106.9	1751.0
9	107.7	1753.6
10	104.7	1765.7
11	107.0	1766.8
12	103.0	1759.3
Average	106.7	1765.2
STDEV	1.43	17.97
% RSD	1.34	1.01

D. EDTA Analyses in Dairy Waste Waters

Around 80 dairy waste waters were analyzed, including wastewater from the processing plants, wastewater during the wastewater treatment processing and dairy effluent discharging into the adjacent river. The concentrations of EDTA varied from 72 µg L^{-1} to 82.7 mg L^{-1}. A typical chromatogram of dairy waste water is shown in Fig. 6. The range of EDTA concentration (72 ~ 261 µg L^{-1}) of dairy effluents discharged into the adjacent river is shown in Fig. 7. Data was from 3 days in August, 3 days in October and 7 days in December 2007. Samples were all 24–hour composite.

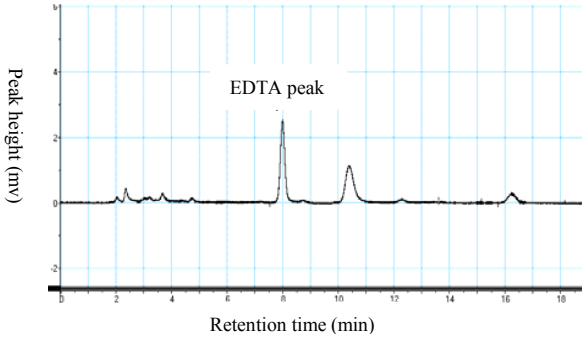

Fig. 6 Typical chromatogram of a dairy wastewater sample

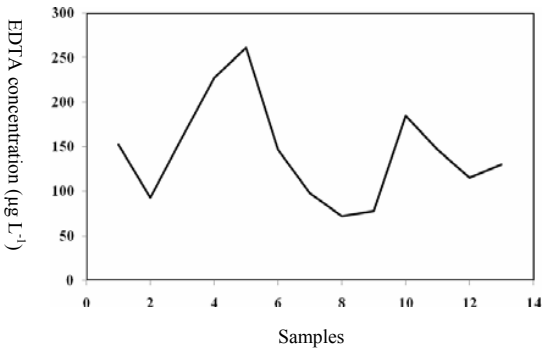

Fig. 7 Concentrations of EDTA in dairy effluents discharged into the adjacent river from a large dairy site

E. Determination of EDTA in Surface Water

Forty eight surface water samples, collected in August and October 2007, were analyzed by the described HPLC method. A typical chromatogram of the surface water sample is shown in Fig. 8. The calibration curve was established daily at the concentration of 0 to 150 µg L^{-1} (0, 10, 20, 50, 80, 100 and 150 µg L^{-1}). Duplicate analyses were undertaken every 10 samples and the spike recoveries of surface water (50 µg L^{-1}EDTA standards) every 20 samples as a quality control during the experiment. Averaged duplicate variability was 8.1 % (n=5) and spike recovery varied from 97 – 107% (n=3). The averaged EDTA concentrations, with one standard deviation, upstream and downstream of the dairy effluent discharge are shown in Fig. 9. This data represents averaged 12 results at each site. It can be seen from Fig. 9 that concentrations of EDTA were increased downstream 60 m of the effluent discharge before the other stream joins in. The change of EDTA contents was not obvious at 10 m downstream as EDTA may not be mixed well with river water yet. The highest concentration of EDTA analyzed at 60 m downstream was 2.7 µg L^{-1}. This was well under the predicted no effect concentration for aquatic environment – 2.2 mg L^{-1}[3].

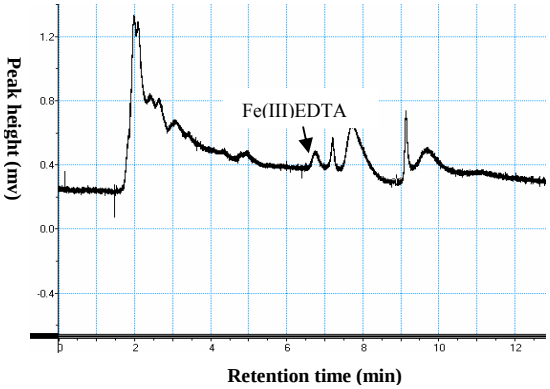

Fig. 8 Typical chromatogram of a surface water sample with 1.7 µg L^{-1} of EDTA

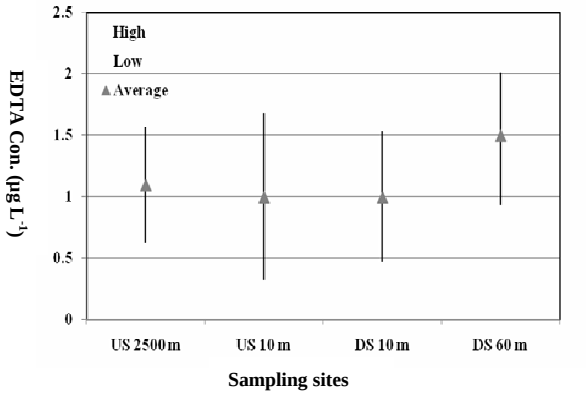

Fig. 9 EDTA concentrations upstream and downstream of dairy effluent discharge in the adjacent surface water

IV. Conclusion

An HPLC-UV analytical method was described to determine EDTA as Fe(III)EDTA using UV detection. The chromatography separation was optimized by adjusting compositions of the mobile phase and flow rate on a C18 reversed-phase column. The possible interferences of nitrate, calcium and magnesium from dairy waste waters were investigated and no interference found. Dairy waste waters with varying EDTA levels between µg L^{-1} to mg L^{-1} were analyzed with an acceptable repeatability (duplicate) and reproducibility (spike recovery). The method detection limit was 5 µg L^{-1}.

The method was applied to determine EDTA in the adjacent surface water into which large amounts dairy effluents (7,000 m^3) were discharged. A contribution of EDTA from the dairy effluent was observed at 60 m downstream before the other stream joined in, but not 10 m downstream of the discharge. Overall EDTA concentrations in the adjacent surface water were less than 3 µg L^{-1}. This was well under the predicted no effect concentration for aquatic ecosystem.

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