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Laser induced resonance energy transfer – a novel approach towards achieving high sensitivity in capillary electrophoresis¹

Part B: Applications for environmental testing

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Abstract

Capillary electrophoresis with laser induced resonance energy transfer detection is used for direct determination of ethylenediamine tetraacetic acid (EDTA), 1,2-diaminopropane-N,N,N',N'-tetraacetic acid (DPTA) and (\pm)-*trans*-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (DCTA) in aqueous samples. The method involves CZE separation of salicylate-Tb-EDTA, salicylate-Tb-DPTA and salicylate-Tb-DCTA ternary complexes at alkaline pH. The highly efficient transfer of energy from salicylate to terbium in these complexes facilitated a sensitive detection. Detection limits in the $1 \cdot 10^{-7}$ M range are easily achieved. A 325 nm HeCd laser is used for excitation and terbium luminescence is monitored at 547 nm. The potential application of this method in analyzing these acids in a complex radioactive waste is discussed.

Keywords: Laser-induced resonance energy transfer; Detection, electrophoresis; Resonance energy transfer; Ethylenediaminetetraacetic acid; 1,2-Diaminopropane-N,N,N',N'-tetraacetic acid; *trans*-1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid

1. Introduction.

One of the main challenges faced by countries generating nuclear waste is the remediation of the radioactive waste stored in numerous underground storage tanks (UST). In the United States alone there are 332 USTs. Two types of storage tanks are prevalent: single shell wall (SST) and double shell wall (DST). Together, these tanks hold more than 100 million gallons of high-level and low-level radioactive waste, very little of which has been

treated and disposed. In the USA this waste is localized at four main sites which include Hanford Site in Richland, Washington, the Idaho National Engineering Laboratory (INEL) in Idaho Falls, Idaho, Oak Ridge reservation in Oak Ridge, Tennessee and Savannah River site in Aiken, South Carolina. The Hanford site alone holds 177 tanks which accounts for over 60% of the total DOE waste. Most of these tanks are SSTs which have exceeded their life expectancy and are leaking [1].

Waste types stored in SSTs have been described by Anderson [2] and Lucas [3]. According to the "tracks radioactive components" (TRAC) study of 1984, several of the 49 waste types discharged to SSTs are hazardous complex concentrates containing

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organic metal chelators, nitrites, nitrates, organic wash, decontamination and neutralized aqueous tributyl phosphate waste [4]. The presence of organic chelators constitutes the organic safety issue. At sufficient concentration these organic contaminants could act as fuel for a violent exothermic reaction in the presence of strong oxidizers such as nitrite and nitrate. Such reactions could generate aerosols that might plug and rupture the high efficiency particulate air filter and release radioactive material into the environment. Analyses have already shown that these reactions could occur if a portion of waste containing sufficient concentration of organic compounds and oxidizer is heated to temperatures above 180°C. The atmosphere in USTs is extremely severe with temperatures ranging anywhere from ambient to 93°C and a tank void-space radiation levels as high as 10 000 rad/h [1]. The 640 MCi of radioactive nuclides are distributed primarily among the transuranic elements and fission products, primarily Sr-90 and Cs-137. This usually is not a concern for DSTs since they contain large quantities of water, but however poses a severe threat for SSTs from which the water is removed due to the risk of underground leakage.

EDTA is one of the most routinely used organic chelators at the Hanford site [5]. In particular the determination of EDTA and its analogues in this matrix presents a unique analytical challenge. The challenge is two fold, (i) no generally accepted method exists for the determination of these chelators, chelator fragments and low-molecular-mass acids in radioactive mixed hazardous waste and (ii) studies with simulated wastes have indicated a rapid degradation of these compounds in such a highly radioactive environment [6,7]. The lack of fluorescence and low molar absorptivity in the visible region obviates the direct application of either fluorescence or absorbance methodologies. The polarity and non-volatility of these chelators precludes direct GC-MS determination. Presently, the determination involves overnight stirring of the samples with water, filtration, evaporation of aqueous leachate and finally derivatization with $\text{BF}_3/\text{CH}_3\text{OH}$ to form methyl esters. After extraction, the chloroform solution of methyl ester is subjected to GC-MS analysis. The goal of this work is to develop a direct, sensitive and rapid method for the determination of EDTA and

its analogues which can later be used for analysis of radioactive waste originating from different SSTs.

Capillary electrophoresis (CE) with laser-induced resonance energy transfer (RET) detection is ideally suited for analysis of these chelators. Polyaminopolycarboxylic acids (PPCA) such as EDTA and its analogues are known to form ternary complexes with lanthanides, specifically Tb^{+3} , in the presence of salicylic acid. These complexes are highly luminescent and stable at alkaline pH [8]. The luminescence in these complexes has been attributed to the intramolecular energy transfer from salicylates to terbium [9]. Since the excitation and emission wavelength for different PPCA complexes overlap, it is not possible to determine individual PPCAs directly in a mixture. Therefore in order to use resonance energy transfer from salicylic acid to chelated terbium for determining different PPCAs, the separation of the respective ternary complexes is of utmost importance. By using a buffer composed of sodium carbonate and salicylic acid we report here for the first time the separation and detection of these ternary complexes by capillary electrophoresis. This not only permits the separation of various analogues of EDTA, but also allows a sensitive and direct on-line detection of these compounds.

2. Experimental

2.1. Instrumentation

CE analysis was performed on Beckman P/ACE 5500 (Beckman Instruments, Fullerton, CA, USA) equipped with SYSTEM GOLD software for data analysis. A LIF detector equipped with 20 nm bandpass filter was used for monitoring terbium ion luminescence at 547 nm. A 325 nm HeCd Series 39 laser (Omnichrome, Chino, CA, USA) was used for excitation. The separation of EDTA and its analogues was performed in a 107 cm (injection to detection) \times 75 μm I.D. \times 375 μm O.D. fused-silica capillary tube (Beckman Instruments). High-pressure injections were made for 5 s and the temperature of the capillary was maintained at $16 \pm 0.1^\circ\text{C}$. The applied voltage was 20 kV and the operating currents were less than 100 μA .

2.2. Materials and methods

Sodium salt of salicylic acid (2,4-dihydroxybenzoic acid), sodium carbonate, ethylenediamine tetraacetic acid disodium salt (EDTA) and terbium chloride (99.99%) were purchased from Sigma (St. Louis, MO, USA). 1,6-Diaminohexane-N,N,N',N'-tetraacetic acid (DHTA), 1,2-diaminopropane-N,N,N',N'-tetraacetic acid (DPTA) and (\pm)-*trans*-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid monohydrate (DCTA) were bought from Aldrich (Milwaukee, WI, USA). The separation was performed in a buffer composed of 0.025 M sodium carbonate and 0.001 M salicylic acid with pH adjusted to 11 with 0.1 M NaOH. The buffers were prepared once a week and filtered through 0.45- μ m nylon syringe filters Waters (Milford, MA, USA). EDTA analogues stock solutions (0.01 M) were made by dissolving the respective compounds in deionized water and were used to prepare $1 \cdot 10^{-3}$, $5 \cdot 10^{-4}$, $1 \cdot 10^{-4}$, $5 \cdot 10^{-5}$, $1 \cdot 10^{-5}$ and $1 \cdot 10^{-6}$ M standards in deionized water. Each standard was run in sets of five to monitor the precision of migration times for each analyte.

The fused-silica capillary was activated by rinsing

it with 0.1 M NaOH for 10 min followed by distilled water for an equal period of time. Before each injection the capillary was equilibrated with run buffer for 3 min. At the end of each run the capillary was washed for 3 min with 0.1 M NaOH followed by deionized water.

2.3. Analysis of EDTA analogues in spiked water samples

Municipal tap water was spiked with each EDTA analogue to give a concentration of $1 \cdot 10^{-5}$ M.

To 1 ml of spiked sample 0.1 ml of 0.01 M TbCl₃ was added and pH adjusted to 11 with 0.1 M NaOH. The solution was centrifuged to remove uncomplexed Tb(OH)₃ and supernatant solution was injected hydrodynamically for 10 s.

3. Results and discussion

Fig. 1 shows the baseline separation of three different ternary complexes by CE and their subsequent detection by laser induced RET. The separation is achieved in less than 30 min and the

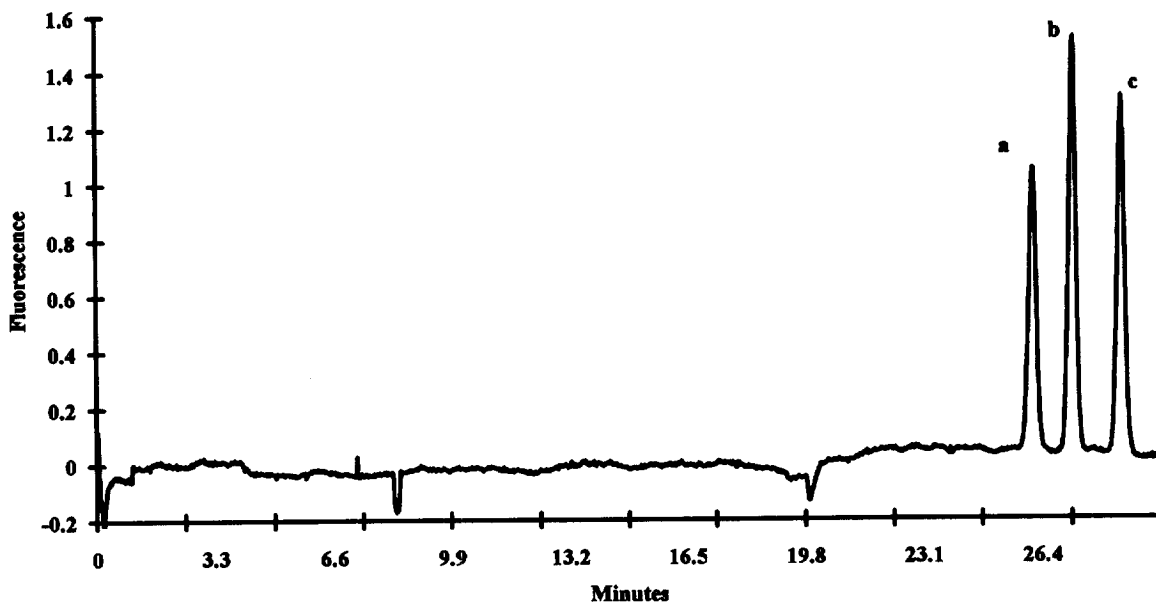


Fig. 1. Separation of three polyaminopolycarboxylic acids, (a) (\pm)-*trans*-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid; (b) 1,2-diaminopropane-N,N,N',N'-tetraacetic acid; (c) ethylenediaminetetraacetic acid.

migration times were highly reproducible with a relative standard deviation of less than 2%. To the best of our knowledge this is the first application depicting the direct detection of different PPCAs in CE. All hitherto published CE procedures involve either indirect absorbance or fluorescence detection giving detection limits in the submillimolar range [10–12]. On the other hand a detection limit (concentration giving $S/N=3$) of $1 \cdot 10^{-7} M$ is easily achieved when laser induced RET is used to detect these compounds.

The energy transfer efficiencies and thereby the detection limits were found to be dependent on (a) pH, (b) concentration of salicylic acid and (c) the chemical structure and stability of lanthanide chelate formed by the different PPCAs. In accordance with earlier reports the terbium ion luminescence was maximum between pH 11 and 12 [8]. Doubling salicylic acid concentration at this pH resulted in a 50-fold decrease in terbium luminescence, this quenching may be due to triplet–triplet annihilation occurring at increased salicylate ion concentrations.

The energy transfer efficiency was also found to be dependent on the structure and stability of the PPCA–Tb complex in alkaline conditions. No ion luminescence was observed in nitrilotriacetic acid (NTA), diethylene triamine pentaacetic acid (DTPA), oxalic acid, citric acid and various amino acid chelates. This is mainly due to the dissociation of these chelates at basic pHs, thus resulting in the precipitation of terbium hydroxide. In acidic or neutral conditions energy transfer is not observed due to protonation of salicylic acid which (i) prevented the formation of ternary complex and (ii) caused a hypsochromic shifts in salicylic acid absorbance maxima. However lack of energy transfer in DTPA and DHTA chelates of terbium cannot be attributed to reduced stability, as these chelators are known to form strong complexes with terbium in alkaline conditions. The absence of $Tb(OH)_3$ precipitate in solutions containing these chelators further confirms the stability of these complexes. It may be speculated that the absence of energy transfer in these chelates is due to the steric hindrance caused by extra functionalities, such as hexane chain, in DHTA and amino acetic acid in DTPA.

Sensitization of Tb-EDTA chelates by salicylate was first demonstrated by Charles et al. [8]. The

Table 1
Linear range and detection limits

Analyte	Linear range	Detection limit
EDTA	$1 \cdot 10^{-6}$ – $0.05 M$	$1 \cdot 10^{-7} M$
DPTA	$1 \cdot 10^{-6}$ – $0.05 M$	$1 \cdot 10^{-6} M$
DCTA	$1 \cdot 10^{-6}$ – $0.05 M$	$1 \cdot 10^{-6} M$

photo physical mechanism as proposed by Crosby et al. [9] involved a radiative excitation of salicylates to the first excited singlet state, followed by the inter-system crossing to the first triplet manifold and subsequent deactivation of salicylate triplet by energy transfer to Tb^{3+} . Table 1 gives the detection limits and linear ranges obtained for different PPCAs. The corrected peak areas vs. concentration, calibration curves were linear over three orders of magnitude with correlation coefficients ranging from 0.9532 to 0.9801. The percentage recovery calculated from the spiked samples ranged from 98 to 110% for the different PPCAs. Table 2 shows the theoretical plates calculated from the equation:

$$\text{Theoretical plates} = 5.54[L_d / (\text{area}_{\text{corr}} / \text{peak height})]^2$$

where L_d is the length to the detector and $\text{area}_{\text{corr}}$ is the corrected area [13]. As shown in Table 2 they range from 166 000 to 205 000.

4. Conclusion

The hundreds of tons of EDTA and its analogues used in defense related activities have generated renewed environmental interest at the Hanford site [14]. The currently used GC–MS procedure is lengthy and involves tedious extraction and derivatization steps. Laser induced resonance energy transfer has been applied for the direct detection of three PPCAs by CE. A sensitivity of $1 \cdot 10^{-7} M$ for these carboxylic acid is easily achieved with no

Table 2
Efficiencies for different polyaminopolycarboxylic acids

Analyte	Theoretical plates = $5.54[L_d / (\text{area}_{\text{corr}} / \text{peak height})]^2$
EDTA	205 316
DPTA	193 917
DCTA	166 794

significant sample manipulation. Due to the unavailability of radioactive samples because of security reasons the method was not tested on real samples, however the feasibility of using laser induced RET for the determination of different PPCAs is clearly demonstrated. We believe that this method is an attractive alternative to the currently used GC–MS methods because it is sensitive, selective, rapid and can be automated or operated through a remote robotic system. The method described is not universal for all PPCAs and cannot be used for NTA, DTPA, DHTA and other mono- and dicarboxylic acids. However, the emission band at 547 nm ($^5D_4 \rightarrow ^7F_5$) for Tb and 616 nm ($^5D_0 \rightarrow ^7F_2$) for Eu are hypersensitive bands which show drastic increase in intensity on complexation with simple bidentate and polydentate ligands; a 165-fold increase in terbium luminescence is observed when Tb-EDTA chelate is excited at 240 nm [15]. Thus CE instruments with laser induced RET detection have the potential of yielding rapid, simple and sensitive assays for environmental samples.

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