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Extraction Study of EDTA Chelate in Upwelling Sea Water

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Summary: Ethylenediamine tetracetic acid (EDTA) is one of the best chelating agents for F_e^{3+} in seawater and plays an important role in the mechanism of phytoplankton growth. In this research work, it was tried successfully to extract EDTA by a liquid-liquid extraction process using acetophenone and benzyl alcohol as solvents at different pH. ^{14}C EDTA Na_2 was also used to make the process successful.

Introduction

Bioloigcal systems of which metals are a part involve the interaction of metal ions with organic chelators [1]. Chelating agents have been shown to be of importance in the growth of phytoplankton, and have been used in the field of medicines to control metal uptake [2]. Naturally occurring organic compounds may act as chelators in the sea [3-5]. It has been found that highly productive waters contain not only a rich supply of inorganic nutrients but also an organic factors which appear to govern the rate of phytoplankton growth. Barber et al. [6-7] suggested that it is the interaction of these chelators with trace metal ions which is responsible for enhanced growth. One of the synthetic chelators, i.e. ethylene diaminetetracetic acid (EDTA) is commonly used as a component of sea water enrichment media for culturing and enhancing the growth. EDTA (10^{-6}M) in addition to nutrient-rich sea water from the north African upwelling region enabled the upwelled water to support rapid phytoplankton growth [6].

Experimental

The work was pursued with the aim of extraction and determination of metal complexes of EDTA in marine systems. The following techniques were used for this purpose. (I). Identification of I.R. spectrum of EDTA in presence of sea salts. (II) Extraction of EDTA and EDTA metal complex by a liquid-liquid extraction process from sea water. (III) Extraction of ^{14}C labelled EDTA by a liquid-liquid extraction process from sea water.

The major difficulties involved with the work are the extreme dilution of EDTA in sea water and the presence of immense quantities of inorganic salts which complicate the extraction. Disodium salt of EDTA (Baker Analysis Reagent) was used throughout the experimental work. Its solubility is very low in those solvents which have low solubility in water e.g. carbon tetrachloride, chloroform, benzene and ethyl acetate. Two solvents benzyl alcohol and acetophenone were selected for extracting the EDTA metal complex from sea water on the basis of their high dielectric constant (13.1 at 20°C and 17.38 at 25°C respectively), low solubility in water and high solubility in ether. Primarily it was tried to extract EDTA from its solution (10^{-2}M) in deionized water. The quantity extracted was enough to analyse. This primary success gave many thoughts for working with sea water.

One litre solution of EDTA Na_2 (10^{-3}M) in upwelling sea water was prepared and pH 7.0 was adjusted by using 0.1N HCl. Benzyl alcohol extractions were carried out three times using a volume of the solvent equal to 1/20th. the volume of the aqueous solution. The benzyl alcohol extracts were combined and rinsed twice with a volume of deionized water equal to 1/15th. the volume of benzyl alcohol to wash the extracted sea salts. Two volumes of ethyl ether were added to the benzyl alcohol extract. It was then again extracted by three extractions with deionized water, volume of 1/6th. total benzyl alcohol volume. These aqueous extracts were collected and rinsed thrice with ether 1/15th. the volume fo benzyl alcohol to remove the traces of benzyl alcohol and lyophilized to dryness. This crude product was extracted with methanol (2 ml). After evaporting the methanol, the extracted material was again extracted with methanol and dried. It was found 0.24 mg. The I.R. spectrum observed on a Perkin Elmer 21 Spectrophotometer was not very illuminating. Most probably the EDTA metal complex in the extracted material was insufficient for an I.R. spectrum.

The acetophenone was used as an extracting solvent in place of benzyl alcohol to extract EDTA metal complex from a solution of EDTA Na_2 (10^{-3} M in upwelling sea water. The experiment was carried out on the identical lines as described in previous case. The extracted quantity and its I.R. spectrum were not encouraging.

Next experiment was conducted to identify the I.R. Spectrum of EDTA Na_2 in presence of sea salts. For this purpose primarily the I.R. spectra of EDTA Na_2 with KBr and sea salts with KBr were observed separately using different ratios of the components. The best possible percentage of the active components were found to be 1.5 and 2.5 respectively for good spectra. The pellets become opaque if the ratios of sea salts and KBr are 100: Zero, 75:25, 50: 50 and 25:75 respectively. In continuation of this experiments, a 5 ml solution of EDTA Na_2 ($5x10^{-3}$M) in upwelling sea water was lyophilized and the remaining white residue was subjected to I.R. spectrum after making a pellet with KBr (white residue 2.5%). The spectrum showed strong absorptions at 1452 and 1415 cm^{-1} and weak absorptions at 1468, 1447 and 1433 cm^{-1} indicating the carboxylate anion stretching frequencies. The dilution of EDTA Na_2 in upwelling sea water was increased. Identification of above mentioned absorptions in the spectra of the white residues obtained after lyophilizing the 5 ml fractions of 10^{-4}M and 10^{-6}M solutions was obscure.

The work further progressed to determine the extracting efficiency of the solvents i.e. benzyl alcohol and acetophenone for EDTA at different pH values using ^{14}C EDTA (10^{-4}M) in upwelling sea water. The pH was adjusted by adding IN HCl or 0.5 N NaOH solution. Each solution was extracted twice with benzyl alcohol equal to 1/10 th. the volume of aqueous solution. Five minutes were given for mixing the aqueous and non-aqueous phases each time. The solutions were left for approximately 24 hours for the separation of the layers. Simultaneously acetophenone was used as an extracting solvent in place of benzyl alcohol and the experiment was performed on identical lines separately using the ^{14}C EDTA technique. The samples (1 ml) were collected from each solution before and after the extraction and also from each extract. 10 ml aquasol was mixed with each of the samples. Two samples (1 ml) as blanks were collected from upwelling sea water and mixed with 10 aquasol. Three standards were prepared; first consists of ^{14}C toluene 50 and 10 ml aquasol; second ^{14}C toluene 50 upwelling sea water 1 ml and 10 ml aquasol. Third ^{14}C toluene 50 solvent 1 ml and 10 ml aquasol. All samples were placed in a liquitmat 220 automatic liquid scintillation spectrometer adjusting the time 10 minutes for individual sample to count the radioactivity.

Table-1: Extracted Quantity of ^{14}C EDTA at different pH by benzyl alcohol.

Medium	pH	I Extraction Quantity Benzyl Alc.(ml)	% ^{14}C EDTA Extracted	II Extraction Quantity Benzyl Alc.(ml)	% ^{14}C EDTA Extracted
Seawater	2.5	10	7.96	10	8.57
Seawater	4.0	10	0.53	10	0.42
Seawater	6.0	10	0.24	10	0.16
Seawater	8.0	10	0.25	10	0.18
Seawater	10.0	10	0.24	10	0.15

Table-II: Extracted quantities of ^{14}C EDTA at different pH by acetophenone

Medium	pH	I Extraction Quantity Acetophenone (ml)	% ^{14}C EDTA Extracted	II Extraction Quantity Acetophenone (ml)	% ^{14}C EDTA Extracted
Seawater	2.5	10	0.0060	10	0.0089
Seawater	4.0	10	0.0155	10	0.0169
Seawater	6.0	10	0.0165	10	0.0179
Seawater	8.0	10	0.0082	10	0.0232
Seawater	10.0	10	0.0390	10	0.0150

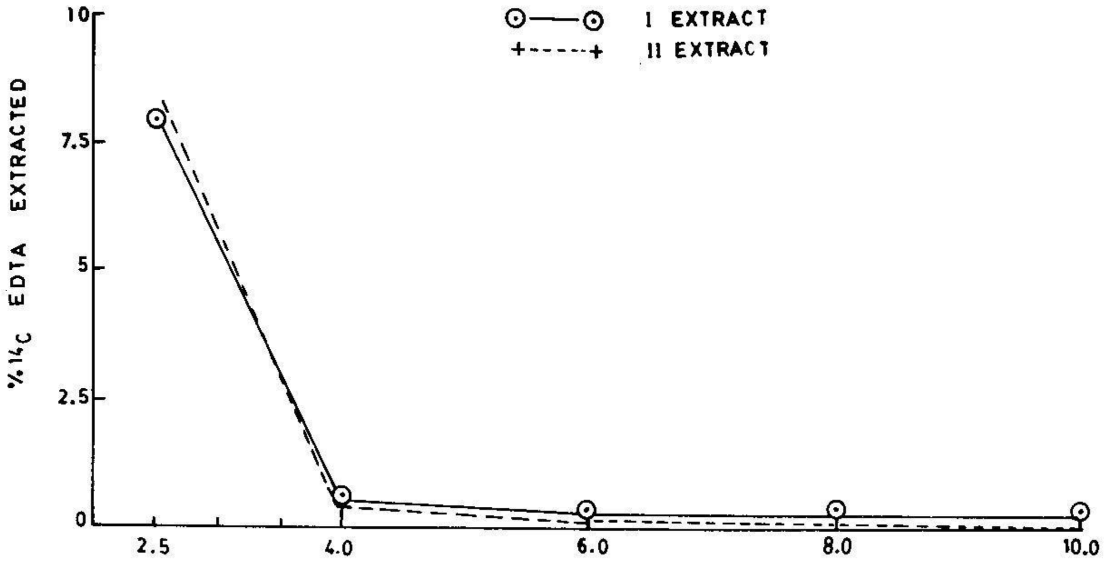

Fig. 1

The results are given in Table-I. The results obtained from the extracting solvent acetophenone are very poor. The data is given in Table II.

The effect of pH on extractability of the solvent for ^{14}C EDTA is very illustrative, shown in Fig. 1. Both subsequent extractions by benzyl alcohol extracted about 8 to 9% EDTA at pH 2.5. Extractability increase as the pH goes down. In view of increasing the extractability of benzyl alcohol, further study was carried out at the pH range 1.0 to 2.5 on the same lines as mentioned before one using the solution of ^{14}C EDTA (10^{-4}M) in upwelling sea water. Mixing and separation time of aqueous and non-aqueous layer were given 5 minutes and 24 hours respectively. The results are tabulated in Table III. The yield is highest at pH 1.0 as shown in Fig. 2.

Discussion

This study provides some of the mechanism that could be applied to extract EDTA and other chelates. Overall results obtained from our research work are very fruitful concerning the extraction of EDTA chelate which is present in extreme dilution in upwelling sea water and no doubt plays a very important role in the growth of phytoplankton. The most interesting informations in Fig. 2 concern the extraction efficiency of benzyl alcohol at pH 2.5 and then a sharp decrease in extraction of EDTA as the pH increases. Further work with reference to pH supported to strong acid media i.e. pH 1.0 for maximum extraction as shown in Fig. 3. This shows that extractability of benzyl alcohol is directly proportional to the acidity of the solution. Parallel investigations were carried out to determine the extraction ability of benzophenone, but it responded very poorly which may be due to its higher dielectric constant than benzyl alcohol. A similar study of other organic solvents with dielectric constants above and below that of benzyl alcohol might lead to a more efficient extraction solvent. Indentification of I.R. absorptions in the residue obtained

Table-III: Extracted quantities of ^{14}C EDTA at different pH by benzyl alcohol

Medium	pH	I Extraction Quantity Benzyl Alc.(ml)	I % ^{14}C EDTA Extracted	II Extraction Quantity Benzyl Alc. (ml)	II % ^{14}C EDTA Extracted
Seawater	1.0	10	10.55	10	11.98
Seawater	1.5	10	8.89	10	9.00
Seawater	2.0	10	7.69	10	8.90
Seawater	2.5	10	5.11	10	5.25

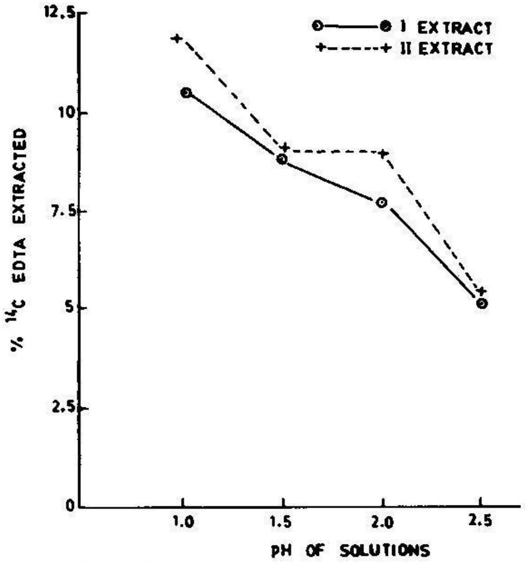

Fig. 2

from the dilution of EDTA Na_2 (10^{-6}M) in upwelling sea water were obscure. In this case it was feared that small quantities of chelator present might easily become trapped in the inorganic salts crystals matricies. This present work succeeded in extracting EDTA as a chelating agent for Fe^{3+} ion in seawater in the presence of immense quantity of sea salts.

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