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EXTRACTION STUDY OF EXTERNALLY-ADDED HYDROXAMIC ACID FROM UPWELLED SEA WATER

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A group of siderochromes, called hydroxamic acids, appear to be synthesized in sea water by phytoplankton. Externally-added hydroxamic acid was extracted successfully from upwelled sea water by liquid-liquid extraction using benzyl alcohol and acetophenone as an extracting solvent at pH 4. The same process gave positive results in the case of fresh water collected from a two years-old fish aquarium.

Key words: Organic acid in sea water.

INTRODUCTION

The presence of naturally occuring organic chelators which enhance phytoplankton growth in marine system has been proposed by several workers [1-4]. On the basis of field observations in upwelling regions, Barber and Ryther [5] and Barber *et. al.* [6] have proposed that eutrophic ecosystems contain soluble organic compounds which enhance phytopankton growth by means of organometallic interactions. However, the extremely low concentration of these chelators in sea water systems has posed problems for their isolation and characterization. In this work, we tried to extract externally-mixed hydroxamic acid from upwelled sea water as well as from fresh water by liquid-liquid extraction process.

Hydroxamic acids have been of interest to organic and analytical chemists for many years primarily due to their ability to form highly coloured Fe^{3+} complexes and to give absorption band maxima around 3000 in the visible region [1]. Ferric hydroxamates exhibit a broad absorption with λ max in the range 425-525 nm [7]. The exact position of λ max is dependent on the structure and concentration of hydroxamic acid, pH, Fe^{3+} concentration and the solvent. A group of siderochromes called hydroxamic acids appear to the ones which are synthesized by phytoplankton. The compounds are named on the basis of the ease of removal of the hydroxylamine protons of the hydroxamate grouping. Naturally occurring hydroxamic acids have been shown to exhibit various kinds of biological activity dependent on their individual structures. Some of these functions include such as antitumour agents, antibiotics, fungistatic agents, growth factors and cell division factors. Precedence of these compounds has been established by Neilands [8] who has extracted hydroxamic acids known as ferric chelating agents from a number of simple organisms, i.e. bacteria, yeast and fungi. Neiland has also suggested that hydroxamic acids may be present in many other simple organisms. Hydroxamic acids have been shown to act as iron-scavaging agents in biological systems and their biosynthesis can be stimulated by placing the organism in an Fe^{3+} deficient medium.

EXPERIMENTAL

The work was carried out to investigate the liquid-liquid extraction process for extracting hydroxamic acid from upwelled sea water along the lines given by Barber *et al.* [5]. For the purpose, one litre solution of hydroxamic acid (ferrioxamine B, 10^{-4}) in upwelled sea water was prepared and pH was adjusted to 4.0 by adding 0.1N HCl. Acetophenone, a solvent of high dielectric constant (17.38 at 25°) and low solubility in water and high solubility in ether, was used as extracting solvent. On identical lines, the extraction was also carried out by another solvent, benzyl alcohol, of lower dielectric constant (13.1 at 20°), as a comparative model at the same pH. [I]. This aqueous solution, adjusted to pH 4.0, was extracted with one of the solvents as mentioned above. (II) The aqueous portion was discarded and the solvent extract was washed with deionised and triple distilled water. [III] Again the aqueous phase was discarded and to the solvent portion ethyl ether was added. It was then extracted with water of the same quality as described above. (IV) Now the solvent-ethyl ether phase was discarded and the remaining aqueous portion was washed with ethyl ether. (V) The ether phase was discarded and the washed aqueous

Table 1. Extraction efficiency data of acetophenone and benzyl alcohol.

Extracting Solvent	Benzyl Alcohol	Acetophenone
Medium	Upwelled sea water	Upwelled sea water
Hydroxamic acid concentration	10^{-4}M	10^{-4}M
pH adjustment	4.0	4.0
Visible λ max	4995	5055
Absorbence	0.24	0.012
Percentage of hydroxamic acid extracted	11.00	0.55

extract was lyophalized to dryness. A crude white powdered material was obtained. To this extracted material, a methanolic solution of $FeCl_3$ (0.001N, 10 ml) was added to convert into its ferric form. The colour of the solution turned to very light red in the case of acetophenone and deep red in the case of benzyl alcohol. Both were filtered through Gelman Glass Fibre Filter (type A) and were subjected to visible spectra. The results given in the Table I show that acetophenone is not a good solvent for the extraction of the hydroxamic acids from aqueous phase phase, while benzyl alcohol proved to be the best one.

Another experiment was carried out in order to study the extraction efficiency by benzyl alcohol at each extraction step, maintaining the pH. 4.0 which is most favourable for extracting purpose. A solution of hydroxamic acid (10^{-4}M and pH 4.0) in upwelled sea water was extracted by benzyl alcohol on the same lines as described in the previous experiment. To each extraction, an equal time of 5 min. was given for shaking the aqueous and non-aqueous phases. The results are given in Table 2 and efficiency curve has been show in Fig. 1

In continuation, an experiment was conducted to determine the presence of hydroxamic acids in fresh water. Two litres of fresh water out of 30 gall. was collected from a two-year old fish aquarium. The water was filtered through Gelman Glass Fibre Filter (type A) and lyophalized. To the remaining white residue 10 ml of $FeCl_3$-MeOH (0.001N) was added. The colour of the solution turned to light red. It was filtered through Glass Fibre Filter (type A) and the visible spectrum was determined. The spectrum indicated the presence of hydroxamic acid (1.72 x 10^{-7}M.) The result is given in Table 3.

Table 2. Extractability of benzyl alcohol for hydroxamic acid.

Medium	No. of extraction	Time for mixing of two phases (min)	Quantity of benzyl alcohol (ml)	λ max	Absorbence	Hydroxamic acid extracted (%)
Upwelled sea water	I	5	50	4950	0.012	5.83
,, ,,	II	,,	,,	5030	0.096	46.60
,, ,,	III	,,	,,	5050	0.014	6.80
,, ,,	IV	,,	,,	5025	0.006	2.91
,, ,,	V	,,	,,	4900	0.016	7.77
,, ,,	VI	,,	,,	5000	0.025	12.13
,, ,,	VII	,,	,,	5050	0.014	6.80

Table. 3. Extraction of hydroxamic acid from fresh water.

Quantity of fresh water	2L
λ max	4550
Absorbence	0.075
Hydroxamic acid present	1.72 x 10^{-7}M

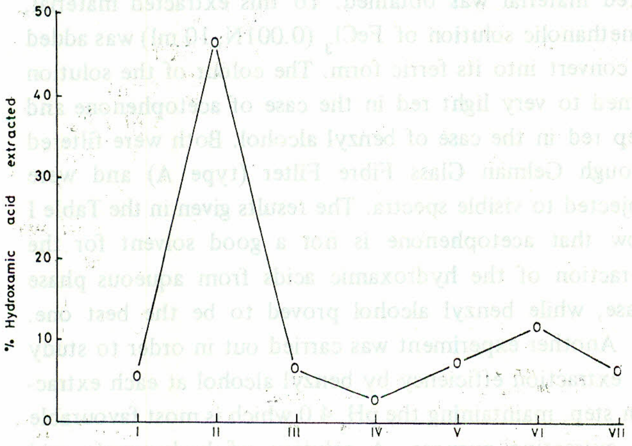
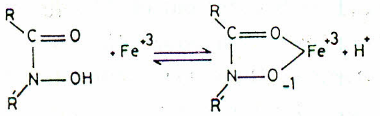


Fig. 1. Number of extractions.

DISCUSSION

Iron is the trace metal which is required in highest concentration by most organisms. Due to its extreme insolubility, as ferric hydroxide $Fe(OH)_3$, at physiological pH, special coordination compounds are needed to keep it in solution. Within the cells, porphyrins fulfil this role of keeping this most required element in the form of chelates at a suitable pH. But apart from prophyrins, there is found at least in aerobic microbial cells, a second major class of iron complexing agents known as siderochromes. A group of siderochromes, e.g. hydroxamic acids, present in sea water are necessary for phytoplankton as they require greater quantitites of iron than other metals for growth [5,9]. The complexed Fe^{3+}, though very tightly bound in hydroxamates, undergoes rapid exchange. This is a commonly observed phenomenon of oxygen ligands on metal ions with the d^5 configuration. The factors, necessary for the best extracting solvent, are low solubility in water, high solubility in ether or any other organic solvent which is equally insoluble in water and of suitable dielectric constant which could efficiently remove the chelators from the aqueous medium. Benzyl alcohol fulfilled these conditions. The hydroxamate ion acts as a bidentate chelate that results in a very stable five-membered ring (10).



The results obtained from this research work are encouraging in a sense that yield is better than the yield of Spencer *et al.* (11). The successful extraction from fresh water is a novel advancement in this field where hydroxamic acids were produced by phytoplankton [6] The data given in the Table 2 suggests that at the first extraction step most of the benzyl alcohol is used up to saturate the solution. The second step is a major one which extracts maximum quantity of the hydroxamic acid. Subsequent extractions do not show gradient change in extracting curve. There occurs the repetition of extractability of the solvent. In the fourth extracting step, again most volume of the solvent is used up by the solution for the purpose as mentioned above. The fifth and sixth steps represent the extraction of the maximum amount of the remaining hydroxamic acids. The poor extractability of the solvent acetophenone, may be due to its too high dielectric constant, insolubility of the dissolved material present in aqeous phase and the structural character of the solvent or other factors which require further investigation in this direction.

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