NUCLEIC ACIDS IN THE DISSOLVED CONSTITUENTS OF SEA-WATER

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ABSTRACT

A method is described to obtain the dissolved constituents of nucleic acid from sea-water by adsorption on *in situ* precipitated $BaSO_4$. The nucleic acid is liberated as hydrolysate in 0·02 N HCl under controlled conditions. The UV spectra, ϵ(P) value, and deoxyribose of the hydrolysate are compared with standard DNA and RNA hydrolysates obtained under similar conditions. The effect of NaCl treatment, amount of *in situ* precipitated $BaSO_4$, temperature, period of hydrolysis, etc., are investigated to obtain pure nucleic acid components from the sea-water system. The recovery of the method is determined using standard DNA and processing the sample under similar conditions.

INTRODUCTION

SEA-WATER contains a variety of organic compounds in the dissolved state in the concentration range of 0·2 to 2·7 mg./l in carbon equivalents. Duursuma,[1] Hood,[2] Skopintsev,[3] Kay[4] and Tatsumato *et al.*[5] have listed the different classes of organic compounds identified in sea-water. Belser[6] obtained indirect evidences for frequent occurrence of uracil and occasional presence of purines in the dissolved state. Hansen *et al.*[7] have observed the presence of nucleic acid in the suspended matter retained on filter-paper. In the course of our investigation, with coastal sea-waters of Bombay, on the role of different organic constituents in the formation of organo-metallic complexes, we have found that sea-water contains a significant concentration of nucleic acid in the dissolved state (probably in the nucleoprotein form). The present paper describes the procedure adopted to separate the dissolved nucleic acid from sea-water and to characterise and estimate the same.

EXPERIMENTS AND OBSERVATIONS

After a number of trial experiments, the following procedure was found to give the best result. Freshly collected sea-water from Bombay Harbour Bay was filtered.* One litre of the filtered sample was kept stirred and to it was added 2·5 ml. of 1% barium chloride slowly in drops (giving 24 mg. *in situ* precipitated $BaSO_4$). Stirring was continued for 2 hours at room temperature (25° C.) and the barium sulphate precipitate was allowed to settle. Supernate centrifuged for 15 minutes at 5800 x g. The precipitate was transferred to a 15 ml. centrifuge tube with distilled water and centrifuged for 15 minutes. The precipitate was washed and centrifuged twice with 10 ml. distilled water and then kept dispersed in 3 ml. of 1 M NaCl for 16 hours by intermittent shaking. The supernate was collected after centrifugation. Residue was washed with 2 ml. of 1 M NaCl, centrifuged and the supernate pooled with the earlier one and filtered. UV spectrum of the 1 M NaCl extract was taken against an appropriate blank (Fig. 1 *a*). To the precipitate was added 5 ml. of 0·02 N HCl and kept for hydrolysis in a water-bath at 100° C. for 3 hours with occasional stirring. The sample was then cooled to room temperature, centrifuged and the hydrolysate filtered. The precipitate was washed with a few drops of distilled water, centrifuged, filtered and added to the main bulk of the filtrate, volume was made upto 5 ml. and the UV spectrum of the hydrolysate taken against an appropriate blank. Figure 1 b shows the maximum and minimum respectively at 260 mμ and 230 mμ. Figure 1 c represents the combined spectrum of the hydrolysate and the NaCl extract. Figure 2 gives the UV spectra of standard DNA† (calf-thymus) and RNA** (yeast) after hydrolysis with 0·02 N HCl under the same conditions as for the sample. Figure 3 gives the UV spectra obtained with standard DNA in 1 M NaCl containing sodium sulphate in concentration equal to that in sea-water and treated similarly as the filtered sea-water sample.

Phosphate content in the hydrolysates was estimated by the method of Meun and Smith[8] and the results are given in Table I.

Carbohydrate contents in the hydrolysates were estimated using the diphenylamine

* 0·22 μ Millipore membrane filters were used in all filtration procedures in the present work.

† Obtained from Sigma Laboratories, USA.

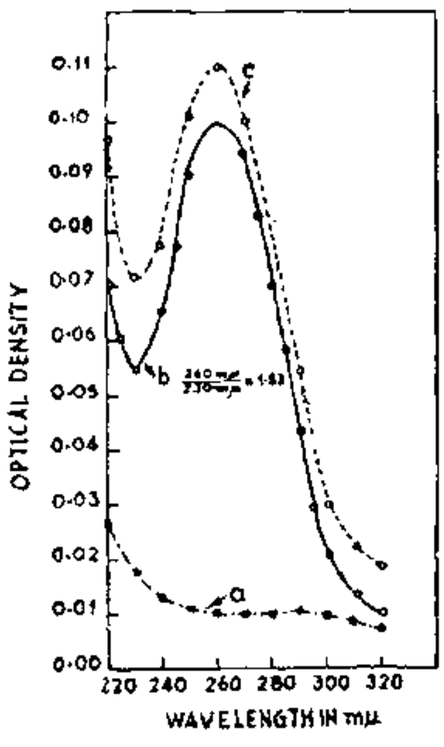
** Obtained from Nutritional Biochemical Corporation, Cleveland, Ohio, USA.

method[9] for deoxyribose. The results are given in Table II.

Discussion

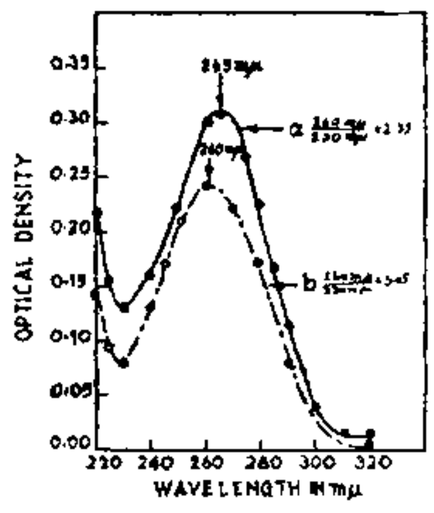
Figure 1 *b* is typical of nucleic acid and compares very well with the standard DNA and RNA in its general features. The supernate after the first precipitation of $BaSO_4$ was treated with barium chloride again to investigate further sorption of nucleic acid, if any left. There was no indication of the presence of nucleic acid in it and Fig. 3 *b* gives the spectrum of the supernate after $BaSO_4$ precipitation in standard DNA solution. At the concentration used, DNA is observed to be picked up about 98% by precipitating $BaSO_4$ under

FIG. 1 - UV SPECTRA OF SAMPLE EXTRACTS FROM PRECIPITATED $BaSO_4$



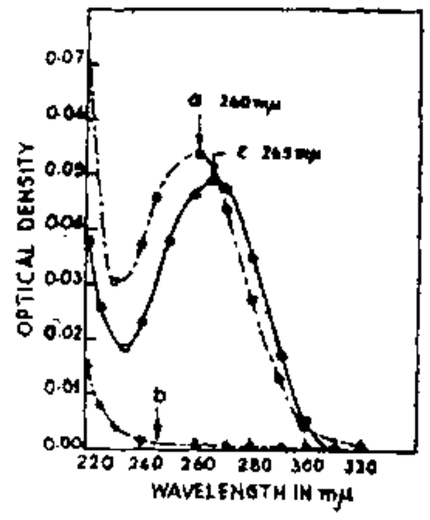
(a) 1 M NaCl EXTRACT
(b) HYDROLYSATE IN 0·02 N HCl
(c) COMBINED SPECTRUM OF NaCl EXTRACT AND THE HYDROLYSATE

FIG. 2 - UV SPECTRA OF STANDARD DNA AND RNA AFTER HYDROLYSIS IN 0.02 N HCl



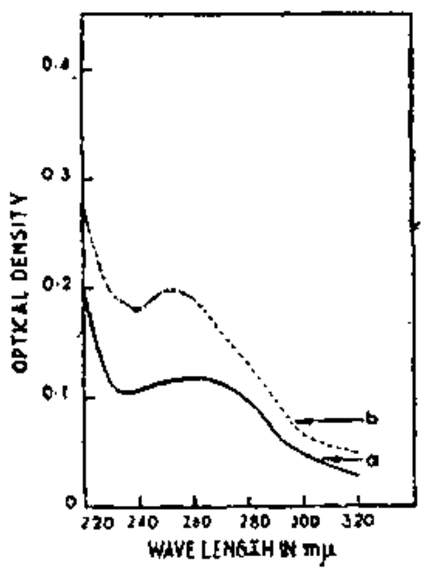
(a) STANDARD DNA (9·52 μg/ml)
(b) STANDARD RNA (9·52 μg/ml)

FIG. 3 - UV SPECTRA OF STANDARD DNA



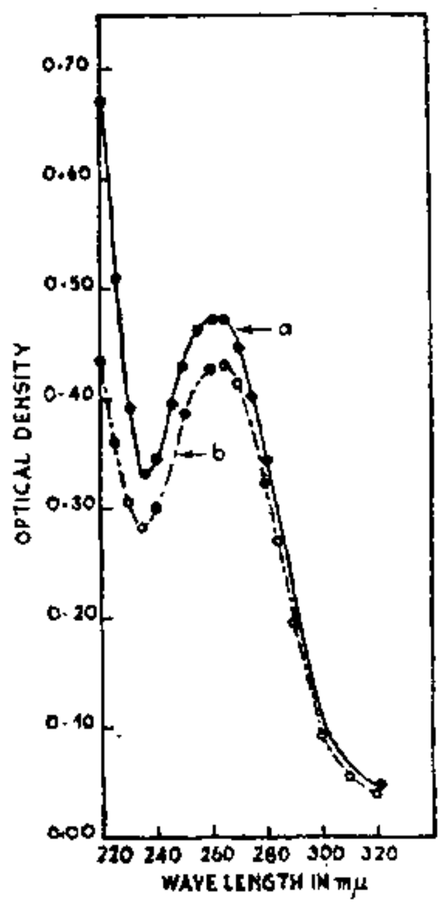
(a) STANDARD DNA IN 1 M NaCl (5·69 μgs/ml)
(b) FILTRATE AFTER $BaSO_4$ PRECIPITATION
(c) HYDROLYSATE IN 0·02 N HCl

FIG. 4 - UV SPECTRA OF THE HYDROLYSATE ($BaCl_2$ USED TO GIVE 48 mgs OF $BaSO_4$)



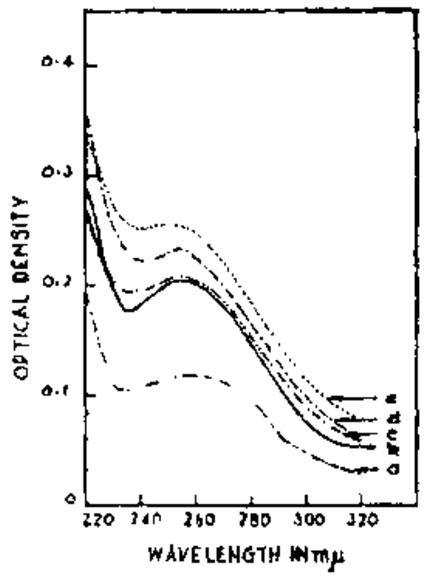
(a) HYDROLYSATE WITH NaCl TREATMENT
(b) HYDROLYSATE WITHOUT NaCl TREATMENT

FIG. 5 - UV SPECTRA OF HYDROLYSATE ($BaCl_2$ USED TO GIVE 96 mgs OF $BaSO_4$) AFTER NaCl TREATMENT



(a) HYDROLYSATE DIRECT
(b) HYDROLYSATE AFTER PURIFICATION WITH OCTYL ALCOHOL - CHLOROFORM (1:5) MIXTURE

FIG. 6 - UV SPECTRA OF THE HYDROLYSATE OBTAINED FROM PRECIPITATED $BaSO_4$ USING DIFFERENT AMOUNTS OF BARIUM CHLORIDE



(a) 48 mgs OF $BaSO_4$ AND 40 HOURS OF 1 M NaCl TREA.
(b) 96 mgs " " " " " "
(c) 144 mgs " " " " " "
(d) 192 mgs " " " " " "
(e) 240 mgs " " " " " "

FIGS. 1–6

neutral condition, whereas under acid condition (0·04 N acid) the adsorption has been found to be poor (~ 50%). The recovery from $BaSO_4$ by the hydrolytic procedure as indicated by UV spectra has been 86% (Fig. 3 c).

The NaCl pretreatment of $BaSO_4$ as described gives hydrolysate agreeing very closely with standard DNA hydrolysate—the ratio of optical density at 260 mμ to 230 mμ for hydrolysates of different samples ranges between 1·8 and 2·2 in agreement with values observed for DNA (*cf.* Ref. 10).

The NaCl extracts in all cases were found positive to biuret test; the extract was not analysed for any other constituent. Figure 4 compares the UV spectra of the hydrolysates obtained by the procedure detailed with and without NaCl pretreatment. The lower ratio of optical density at 260 and 230 mμ for the hydrolysate without NaCl treatment and high absorption values are indicative of protein contamination of the hydrolysate. Figure 5 *a* gives the UV spectrum of nucleic acid obtained from 1 litre of filtered sea-water, using barium chloride to give 96 mg. of $BaSO_4$. The high absorption in the low wavelength region indicated contamination and this sample on shaking with octyl alcohol-chloroform mixture (1 : 5) and removal of the organic phase has the absorption spectrum as in Fig. 5 *b*. NaCl treatment has been observed to be critical in ensuring the retention of only the nucleic acid fraction on $BaSO_4$ and removal of other constituents. Figure 6 gives UV spectra of the hydrolysates obtained from $BaSO_4$ precipitated in sea-water with different quantities of barium chloride The high UV absorptions are associated with high pick-up of interfering dissolved organic matter in the sea-water. The recovery obtained in the hydrolysate from large quantities of $BaSO_4$ has also been observed to be poor. The optimum precipitation of 24 mg. of $BaSO_4$ from one litre of sea-water was derived from these observations. Further, it was necessary to ensure a constant temperature at 100° C. to obtain the optimum hydrolytic product. Longer period of hydrolysis progressively changes the UV characteristics of the hydrolysate.

ε (P) values at 260 mμ calculated, using Figs. 1 *b*, 2 *a* and 2 *b* and the data in Table I, are given in Table III. They ranged from 8150 to 11100 in good agreement with the values obtained for the standard nucleic acids.

Table I

Phosphorous content in the hydrolysate

Sea-water sample collected on	Phosphorous content μg./ml. of hydrolysate	Phosphorous content μg /ml. calculated from the DNA content in the hydrolysate
25–6–1970	0·3685	0·3804
14–7–1970	0·2996	0·2632
21–7–1970	0·6852	0·4944
4–8–1970	1·6150	1·6530

Standard DNA (9·52 μg./ml.) = 0·9763 μg./ml.

Table II

Deoxyribose nucleic acid in the hydrolysate

Sea-water sample collected on	Deoxyribose by diphenylamine method in units of μg. of DNA
25–6–1970	18·55
14–7–1970	13·44
21–7–1970	24·12
4–8–1970	80·63

Table III

ε (P) value of hydrolysates

Sea-water sample collected on :	ε (P) value at 260 mμ
25–6–1970	11730
14–7–1970	10250
21–7–1970	9020
4–8–1970	9046
Standard DNA (9·52 μg./ml.)	9552

The results of deoxyribose estimation, given in Table II, are expressed in equivalents of μg. of DNA by comparing the sample readings with that of the standard nucleic acid. The values thus can also be read as the μg. of DNA present in one litre of the sea-water samples collected on different dates. The DNA concentration given in Table II was used to calculate the phosphorous content in the hydrolysate and the data are presented in the third column of Table I. The calculated values are in good agreement with the values obtained by direct estimation except for one sample. Orcinol method[9] was used in an attempt to estimate the pentose content. The results obtained were 2–3 times higher than the deoxyribose content and as such this could not be correlated with the phosphorous estimation. It is possible that *in situ* precipitated $BaSO_4$ while absorbing nucleic acid, adsorbs also the polysaccharides present in the sea-water filtrate and as such RNA content, if any, in the sea-water collected on 21st

July 1970 could not be made. Further work is in progress.

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ON SOME INTERSTITIAL FAUNA IN THE MARINE SANDS ON INDIAN COAST

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AMONG the various groups of interstitial fauna of marine sands, the ostracods are little known due to their meagre representation in the habitat. Likewise information on the psammophilous halacarid fauna is limited, except for the work carried out on European[1] and North American[2] coasts. The only report of the fauna on Indian coast is that by Chandrasekhara Rao and Ganapati[3] on the beach sands of Waltair Coast. During various faunistic surveys undertaken by the Zoological Survey of India, the author had opportunity to study collections of the fauna from different areas on Indian coasts, including the Andamans. The present paper is concerned with two ostracods (Part I), and the salt-water mite *Halacarus anomalus* (Part II).

Part I. On the Occurrence of Two Interstitial Ostracods (Crustacea) on Indian Coast

Among the ostracods encountered, the occurrence of two European species on Indian coast is interesting and the following is a brief report on them.

Sub-order : Podocopa.
Family : Cytheridae.
Sub-family : Microcytherinae.
Genus : *Microcythere* Muller.

Microcythere subterranea Hartmann

Material.—1 ♂ + 3 ♀, Puri (Orissa), 16th December 1966 ; 1 ♀, Konarak (Orissa), 18th December 1966 ; 2 ♂ + 7 ♀, Waltair (Andhra), 16th November 1968 ; 3 ♂ + 8 ♀, Mayabunder (N. Andaman), 29th March 1969 ; 2 ♀, Cheriatapu (S. Andaman), 6th April 1969 ; 1 ♂ + 5 ♀, Car Nicobar, 13th March 1969 (all collected by G. C. Rao); 1 ♂, Mangalore (Mysore), 17th December 1966 (collected by A. N. P. Ummerkutty).

Remarks.—Hartmann[4] described the species from the intertidal sands on French coast of the Mediterranean Sea. Later, Renaud-Debyser[5] reported the species at Arcachon on the Atlantic coast. This species has now been recorded for the first time on the west coast and Andamans. The specimens collected on Indian coast agree fairly well with the description and figures given by Hartmann. The Indian forms measure 0·18-0·20 mm. in length and 0·06-0·07 mm. in width. The terminal segment of the antennule bears a modified seta, not reported in the type.

Sub-family : Loxoconchinæ.
Genus : *Microloxoconcha* Hartmann.

Microloxoconcha compressa Hartmann

Material.—2 ♂ + 5 ♀, Waltair (Andhra), 16th November 1968 ; 2 ♂ + 7 ♀, Mayabunder