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# NUCLEIC ACID IN THE DISSOLVED CONSTITUENTS OF SEA WATER*

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ABSTRACT

Detection of nucleic acid in the dissolved constituents of sea water has recently been reported by the authors. The dissolved nucleic acids are carried down by *in situ* precipitated $BaSO_4$. In the present study for optimisation of recovery of nucleic acid hydrolysate, investigations havebeen carried out on effects of: quantities of *in situ* precipitated and added $BaSO_4$, sodium chloride treatment, temperature and period of hydrolysis and the strength of acid used for hydrolysis. Characteristics of the nucleic acid hydrolysate isolated by adopting the optimum conditions so obtained, are compared with those of standard DNA (calf-thymus). Sea water collected from Bombay Harbour Bay has been found to contain about 20 $\mu g$ DNA per litre.

## INTRODUCTION

THE work presented here is in continuation of the studies on 'Nucleic Acids in the Dissolved Constituents of Sea Water'. A method to isolate the dissolved constituents of DNA from sea water by absorption on *in situ* precipitated $BaSO_4$, treatment of the precipitate with sodium chloride and then hydrolysis of the absorbed material on $BaSO_4$ with 0.02 N HC1 at 100°C for three hours for the separation of the constituents of nucleic acid and comparison of the characteristics of the hydrolysate with those of standard DNA (calf-thymus) have been described earlier (Pillai and Ganguly, 1970).

In the present work, systematic studies were carried out to establish optimum conditions required for the isolation and purification of the hydrolysate. The characteristics of the hydrolysate are compared with standard DNA processed under similar conditions. Fig. 1 compares the UV spectra (Pillai and Ganguly, 1970) of the hydrolysate sample from sea water with that of standard DNA.

## EXPERIMENTAL PROCEDURE

The experimental procedures given above have been described in detail by Pillai and Ganguly (1970). All experiments were performed with one litre sample of sea water collected from Bombay Harbour Bay and filtered through 0·22 $\mu$ Millipore membrane filter.

## RESULTS AND DISCUSSION

The difference in the effectiveness of *in situ* precipitated and freshly added $BaSO_4$ can be seen in Fig. 2. Under identical conditions, *in situ* precipitated

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$BaSO_4$ resulted in higher recovery of dissolved nucleic acid from sea water than that with the same quantity of freshly precipitated $BaSO_4$ added to the duplicate

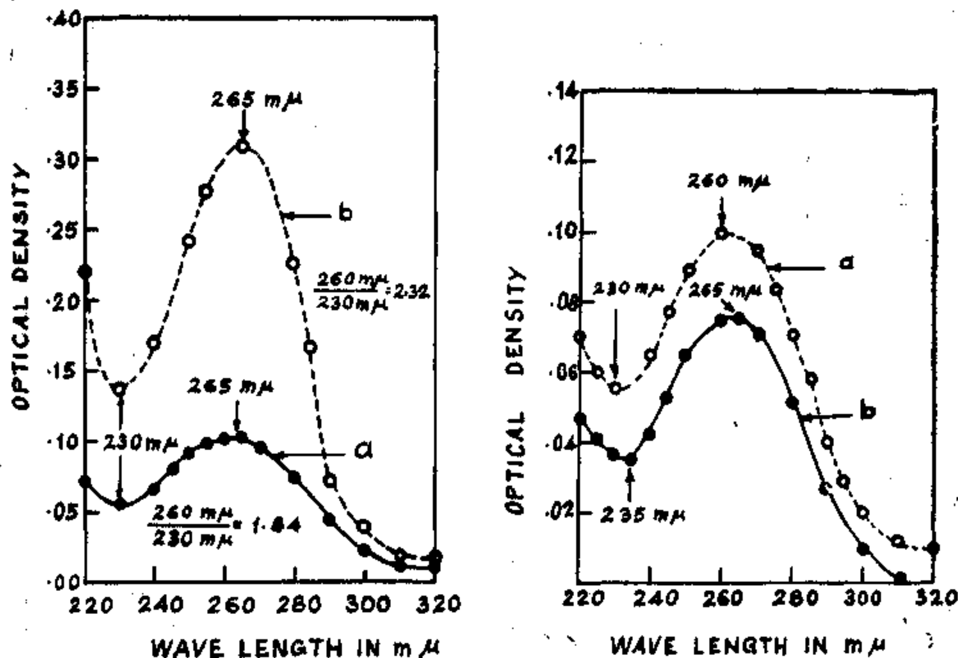



Fig. 1. (Left) UV spectra of the Hydrolysate sample from sea water and standard DNA. a. *in situ* precipitated $BaSO_4$ Hydrolysate in 0·02 N Hcl, and b. standard DNA (9·52 μg/ml) Hydrolysate in 0·02 N HCl.

Fig. 2. (Right) UV spectra of the Hydrolysates obtained from *in situ* precipitated and freshly added $BaSO_4$. a. 24 mgs *in situ* precipitated $BaSO_4$ and b. 24 mgs freshly added $BaSO_4$ precipitate.

sample of sea water. The effect of different quantities of *in situ* precipitated $BaSO_4$ is given in Fig. 3. The recovery of DNA as hydrolysate has also been found to be poor when large quantities of $BaSO_4$ are precipitated (Curve 'e' Fig. 3). As can be observed from the curves, the recovered hydrolysates are also contaminated with other products when large quantities of $BaSO_4$ are precipitated. In Fig. 4, one of the hydrolysates obtained from $BaSO_4$ (96 mgs precipitated in a litre of sea water) when shaken with octyl alcohol-chloroform mixture (1:5), the aqueous phase was observed to indicate a better spectrum of nucleic acid as shown in Fig. 4b. The precipitation of 24 mgs of $BaSO_4$ per litre of sea water, by the progressive addition, with constant stirring, of the requisite quantity of dilute barium chloride solution was observed to be optimum for carrying down the nucleic acid quantitatively with minimum of the other organic constituents.

*In situ* precipitated $BaSO_4$ has to be shaken with 1 M NaCl before hydrolysis and this treatment has been observed to be critical in ensuring the retention of only the nucleic acid fraction on $BaSO_4$ and removal of other absorbed organic constituents. The effect of time of contact in NaCl treatment was studied for 2 hours, 4 hours and 16 hours. The treated $BaSO_4$ hydrolysed under identical conditions.

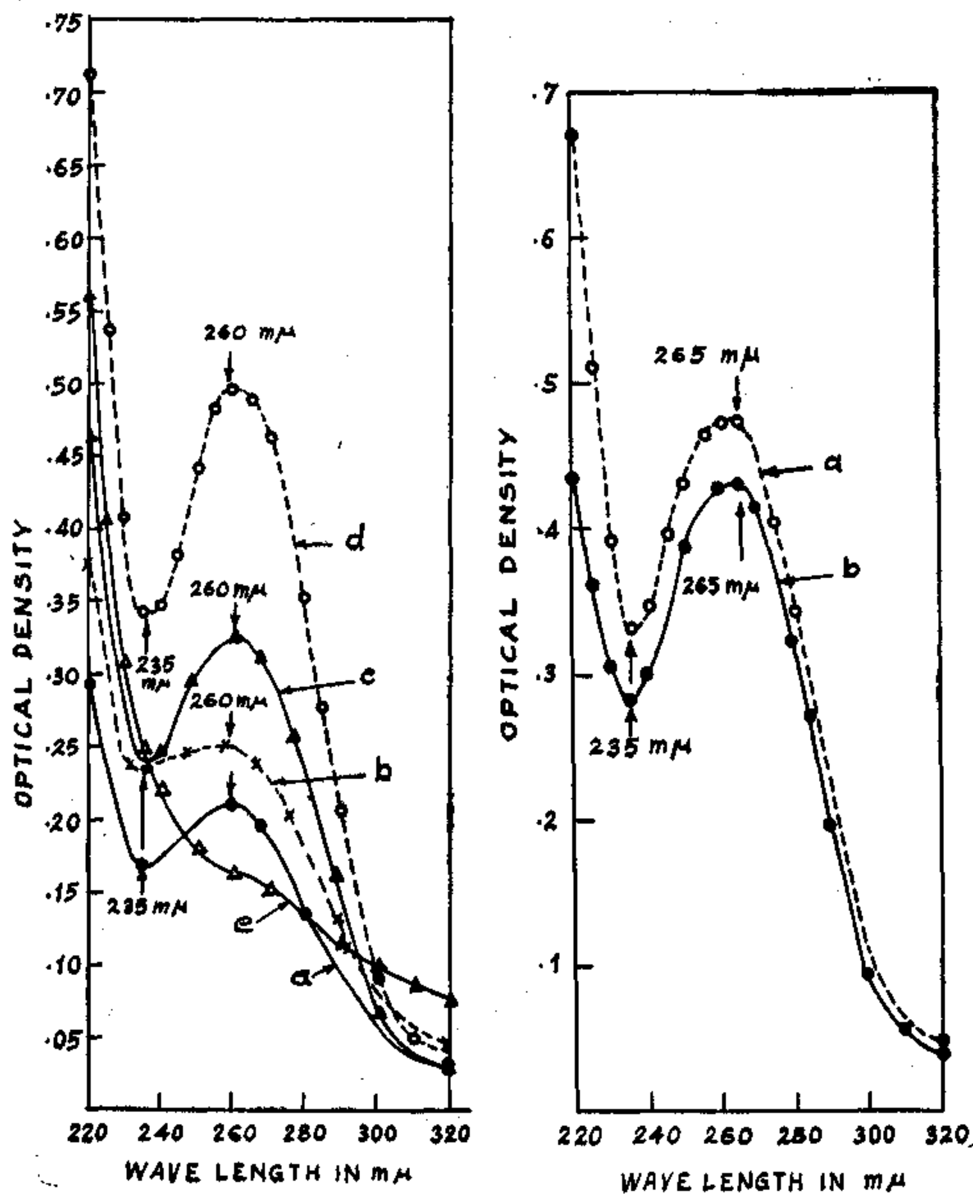



Fig. 3. (Left) UV spectra of the Hydrolysates obtained from *in situ* precipitated $BaSO_4$ using different amounts of Barium chloride. a. 24 mgs of $BaSO_4$ and 16 hrs of 1 M Na Cl treatment; b. 48 mgs of the same; c. 72 mgs of the same; d. 96 mgs of the same and e. 7.64 gms of the same.

Fig. 4. (Right) UV spectra of Hydrolysate ($BaCl_2$ used to give 96 mgs of $BaSO_4$) after Na Cl treatment. a. Hydrolysate direct and b. Hydrolysate after purification with octyl alcohol—chloroform mixture (1 : 5).

The UV spectra of recovered hydrolysates are given in Fig. 5. It was noticed that prolonged NaCl treatment removes the proteneous material (NaCl exrtact positive to biurat test) and the hydrolysate was found to be almost free of protein. The NaCl extract was not analysed for any other constituent.

Hydrolysis of $BaSO_4$ was carried out at 25°, 65°—95° and at 100°C. At room temperature, there was no recovery and a satisfactory recovery was obtained when the hydrolysis was carried out at constant temperature of 100°C (Fig. 6).

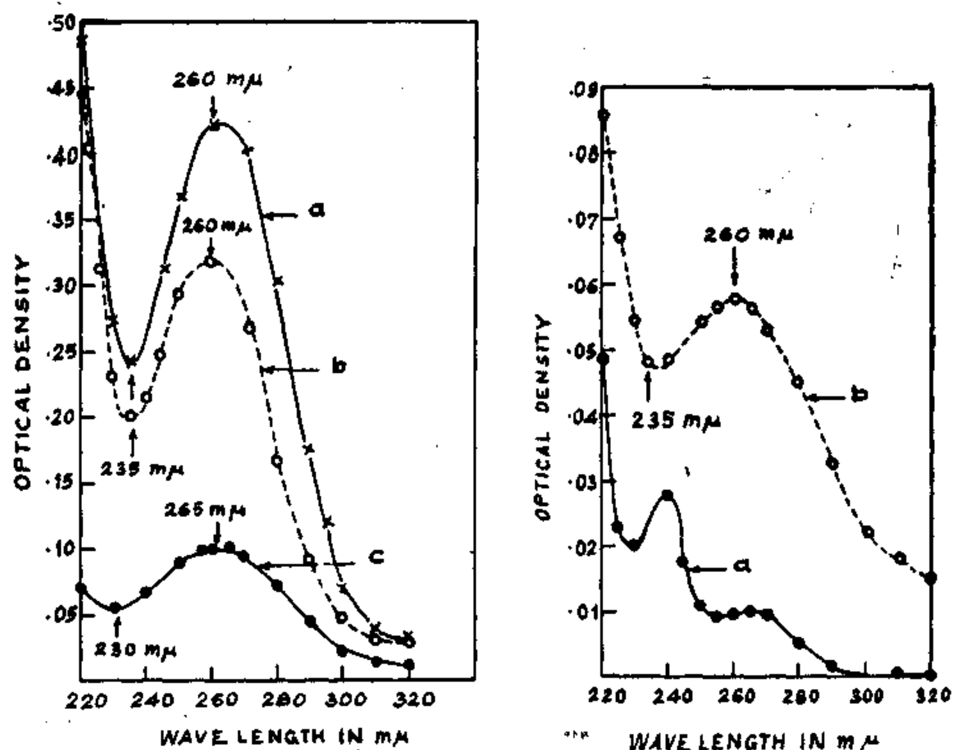
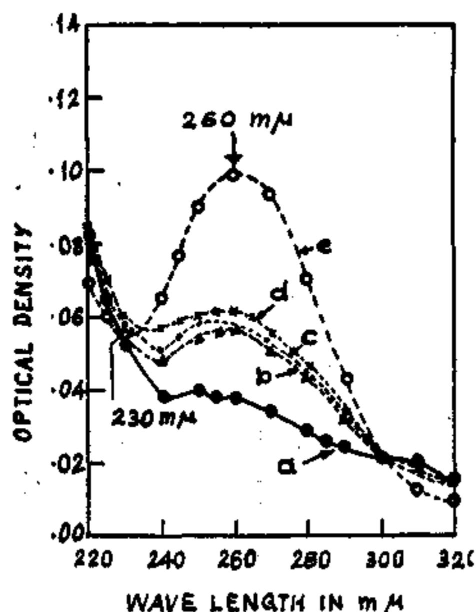



Fig. 5. (Left) UV spectra of the Hydrolysate obtained from $BaSO_4$ treated with different period of contact with 1 M NaCl. a. Hydrolysate obtained from 24 mgs $BaSO_4$ after 2 hrs. of treatment with 1 M NaCl. b. the same after 4 hrs. treatment and c. the same after 16 hrs. treatment.

Fig. 6. (Right) UV spectra of the Hydrolysate obtained at different temperatures (Hydrolysis time 2 Hrs.). a. Hydrolysate obtained from 65°-95°C and b. Hydrolysate obtained at 100°C.

The effect of duration of hydrolysis on recovered hydrolysate was studied and the UV spectra obtained is given in Fig. 7. Long periods of hydrolysis progressively changes the characteristics of the hydrolysates. This was the case when the period of hydrolysis was extended beyond 3-4 hours. The optimum hydrolysis time of 3 hours was adopted where the characteristics of the hydrolysate were found to correspond very well with those of standard DNA.



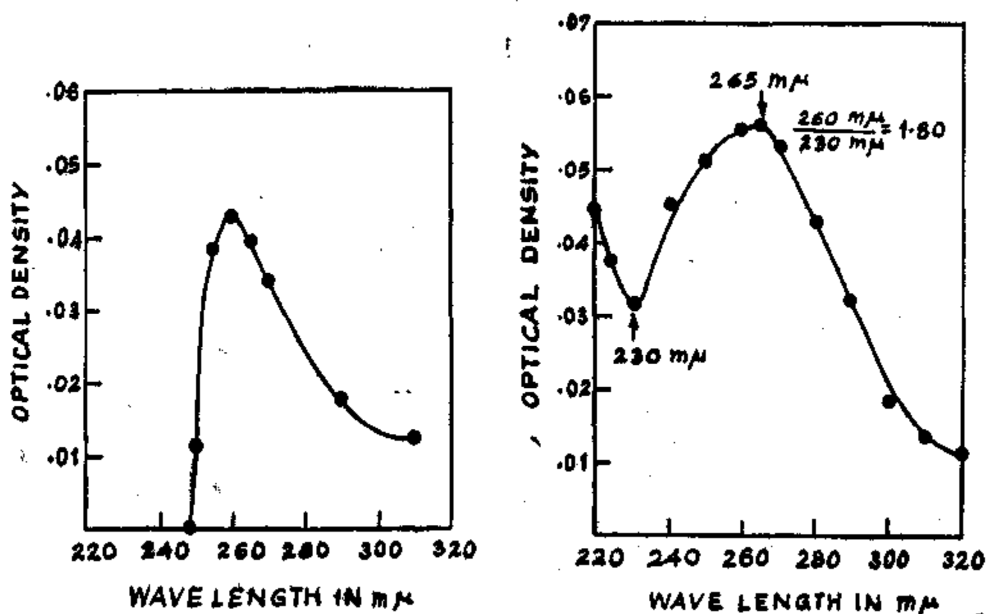




Fig. 7. (Top) UV spectra of the Hydrolysate (from 24 mgs $BaSO_4$) obtained from different period of hydrolysis. a. 15 minutes, b. 30 minutes, c. 1 hr. d. 2 hrs. and e. 3 hrs. hydrolysis at 100°C.

Fig. 8. (Left) UV spectrum of the Hydrolysate obtained from DNA absorbed $BaSO_4$ during hydrolysis with 2 N HCl for 2 hrs. at 100°C.

Fig. 9. (Right) UV spectrum of the Hydrolysate obtained by adopting the optimum parameters.

The strength of acid required for hydrolysis was found to be critical. High concentration of acid was found to destroy the characteristics of the hydrolysate.

TABLE 1. *Mode of Operation*

| Parameters | Optimum conditions |
|---|---|
| Volume of sea water (0·22 μ filtered) | .. 1 litre |
| Amount of *in situ* precipitated $BaSO_4$ | .. 24 mg |
| Period of stirring (at room temperature) | .. 2 hours |
| Period of treatment with 1 M NaCl | .. 16 hours |
| Temperature of hydrolysis | .. 100°C |
| Period of hydrolysis | .. 3 hours |
| Strength of acid used for hydrolysis | .. 0·02 N HCl |

Fig. 8 gives the UV spectrum for the case where DNA absorbed on $BaSO_4$ was hydrolysed with 2 N HCl. The optimum concentration of 0.02 N HCl was derived after a number of trial experiments.

TABLE 2. *Characteristics of the Hydrolysate*

| Parameters | Sample | Standard DNA |
|---|---|---|
| Maximum absorption at | 265 mμ | 265 mμ |
| Minimum absorption at | 230 mμ | 230 mμ |
| Ratio 260 mμ/230 mμ(8) | 1·80 | 2·32 |
| DNA content from diphenyl (3) amine estimation | 20·40 μg/l | 9·52 μg/ml |
| Phosphorus content (4) per ml of hydrolysate | 0·1880 μg | — |
| Phosphorus content μg per ml calculated from the DNA content of hydrolysate | 0·2091 μg | — |
| ε(P) value at 260 mμ | 9061 | 9552 |

Table 1 gives the optimum conditions obtained from the above experiments. Adopting these conditions, a sample of sea water collected on 25th November, 1970, was analysed for nucleic acid. Fig. 9 gives the UV spectrum of the hydrolysate. Table 2 gives the characteristics of the hydrolysate and DNA hydrolysed under similar conditions.

The dissolved DNA contents per litre of sea water collected from Bombay Harbour Bay on different dates are given in Table 3.

TABLE 3. DNA *Content μg per litre of sea water*

| Sea water collected on | DNA content μg per litre |
|---|---|
| 25.6.1970 | 18·55 |
| 14.7.1970 | 13·44 |
| 21.7.1970 | 24·12 |
| 15.11.1970 | 20·40 |

Work on the presence of RNA and the base composition of the nucleic acids is in progress.

REFERENCES

PILLAI, T. N. V. and GANGULY, A. K. 1970. Nucleic acids in the dissolved constituents of sea water. *Curr. sci.*, **22**, Nov. 20.