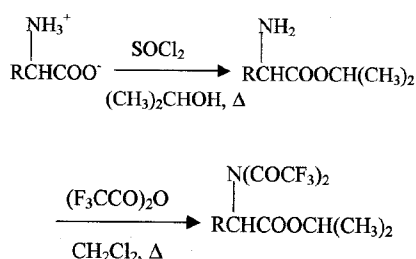


Aminostratigraphy of sediments of the SW Bay of Bengal

Amino acid enantiomeric (D/L) ratio is a stratigraphic tool of Recent sediments. The method which came into being 35 years ago¹, is not a precise dating tool like, for example, the radiocarbon method, as the extent of racemization may not wholly depend on time. Several environmental factors like temperature, pH, bacteria, minerals and water content of the ambient medium affect the racemization rate². Moreover, the quantification of the enantiomeric (D and L) components is a tedious process involving the preparation of GC/HPLC-separable derivatives of amino acids from the sediment hydrolysates. Yet, the global community of earth scientists, on the lookout for multiple palaeo proxies, has continued to pay attention to this method, as it offers some special advantages under specified conditions³⁻⁶, and is relatively inexpensive.

Hence, we wanted to perform the amino acid enantiomeric estimation on Indian marginal sediments. We followed an amino acid derivatization procedure involving the following sequence of reactions in which the *N*-trifluoroacetyl isopropyl esters are formed⁷.



However, by adopting the reaction conditions as reported by Goodfriend⁷, we could not get reproducible results even after repeating the reaction under several combinations of conditions. We realized the problem was with the first step. Instead of heating the sample with the reagents in sealed ampoule when high pressure is developed, we performed the reaction in two sub-steps—first preparing the acid chloride with SOCl_2 (only) by gentle (80°C) reflux (30 min) under open dry condition and then immediately treating the reactive intermediate with anhydrous isopropyl alcohol in the same tube in sealed condition (80°C, 15 min). The second step of

synthesis is as reported by Goodfriend⁷. The residue thereof, in CH_2Cl_2 was passed over SiO_2 gel for purification before injection onto the gas chromatographic column. Our GLC (HP 6890, Chirasil L Val column, NPD) conditions are: He carrier gas, temperature programme of 100°C (hold 10 min), 4°C/min rise up to 140°C (hold 1 min), 10°C/min rise up to 180°C (hold 10 min). The retention times of aspartic acid ester are 18.803 (D acid) and 19.011 (L acid) minutes. A standard error of $\pm 1.5\%$ (5 determinations) was achieved in the D/L ratio, with standard D and L aspartic acids in the range of zero to 1 mg taken for each determination.

For the sediment samples also, the derivatization procedure is identical. However, an additional extraction cum hydrolysis step is applied before derivatization. It involves 6N HCl treatment of the sediment at 105°C for 22 h in a sealed tube when the proteins are converted to individual amino acids⁸, followed by removal of Ca^{2+} with HF. The (gravity) core (445 cm long) sediments used in the present study are from 08°05.090'N, 078°35'E, collected on-board ORV *Sagar Kanya* (cruise no. 157) on 30 September 2000 at a water depth of 1395 m. The sectional sediment samples were previously deep-frozen on-board the ship and frozen dry by a freeze dryer in the shore laboratory to powders, in order to prevent artifacts. Core sediment samples, previously dated by radiocarbon method and which could probably have been made available to us by other laboratories could not be used as standards due to the poor conditions under which they were processed/preserved.

The D/L ratio of aspartic acid of coarse fraction (+ 25 μ) of ten sediment samples (Table 1) analysed representing the entire core length is shown in Figure 1. The ratio gradually increases downcore. The increase of D/L ratio with depth is significantly uniform in the three depth ranges of the surface layer (up to 46.5 cm by extrapolation), the middle layer extending up to 280.5 cm and the bottom layer below 280.5 cm.

Among the above ten samples, three (222, 67.5 and 352 cm) were subjected to age determination by the radiocarbon method. Benzene was synthesized from

the coarse fraction (+ 25 μ) in three steps, namely (i) liberation of CO_2 , (ii) reaction of CO_2 with acetylene, and (iii) trimerization of acetylene. Residual specific ^{14}C radioactivity was measured following liquid scintillation spectrometry method⁹. It is assumed that the sample CO_2 has $\delta^{13}\text{C} = 0\text{‰}$. The conventional radiocarbon ages (in yr BP) were converted into calendar ages by using the calibration program CALIB4.0 (ref. 10), assuming reservoir age to be 402 years (global value). The marine calibration data are not available beyond ~20,760 ^{14}C year BP; therefore for the older 352 cm sample, a simple linear calibration: cal age BP = 1.168 × (^{14}C age BP) has been used to get an approximate age calibration¹¹.

The aspartic acid racemization rate constant K was computed from the equation¹²⁻¹⁴:

$$\ln \left[\frac{1 + (D/L)}{1 - (D/L)} \right]_t - \ln \left[\frac{1 + (D/L)}{1 - (D/L)} \right]_{t=0} = 2kt, \quad (1)$$

where t is time in years BP given by radiocarbon dating. Its value is $0.52 \times 10^{-5} \text{ yr}^{-1}$ and $0.11 \times 10^{-5} \text{ yr}^{-1}$ in the middle (46.5 to 280.5 cm) and bottom (280.5 to 390.5 cm) horizons, respectively. A value of $4.4 \times 10^{-5} \text{ yr}^{-1}$ is obtained for the upper (surface) layer, calculated by extrapolation (since no radiocarbon date is available in this layer). Working on the bulk foraminifera of the coarse fraction (+ 250 μ) of the deep Western equatorial Pacific sediments (water depths: 3604 and 2226 m), Harada *et al.*¹² reported aspartic acid D/L ratios similar to the values in the present study. Their average aspartic acid racemization constant is $0.84 \times 10^{-5} \text{ yr}^{-1}$ in the upper sediments and $0.18 \times 10^{-5} \text{ yr}^{-1}$ (i.e. less by a factor of ~5) in the lower sediments, also as in the present study. The inflections in the profile of D/L ratio, which is a common feature of most studies, are attributed to changes in the sedimentary environment. The older (bottom) sediments are characterized by more stable organic matter (bound amino acid/peptide) that resists racemization, while the upper (recent) sediments are characterized by reactive organic matter in which the (free) amino

Table 1. Aspartic acid racemization in sediments

Depth (cm bsf)	D/L ratio of aspartic acid	Racemization constant ($\times 10^{-5} \text{ yr}^{-1}$) ^s		D/L age range (yr)	Age-calibrated ¹⁴ C age (yr BP)
		Range	Mean		
26.5	0.0625	4.33–4.55	4.44	1498–1476	–
32.0	0.0826	4.33–4.55	4.44	1981–1922	–
67.5	0.1434	0.508–0.526	0.517	4886–4826	4915–4651
110.5	0.1630	0.508–0.526	0.517	8836–8641	–
180.5	0.2017	0.508–0.526	0.517	16,718–16,253	–
222	0.2358	0.508–0.526	0.517	19,372–18,817	19,408–18,652
250.5	0.2467	0.508–0.526	0.517	23,768–23,062	–
280.5	0.2606	0.508–0.526	0.517	28,971–28,087	–
352	0.2738	0.105–0.122	0.1135	42,509–39,739	41,100 \pm 1400
390.5	0.2857	0.105–0.122	0.1135	54,804–50,321	–

–, Not available; ^sIn successive layers.

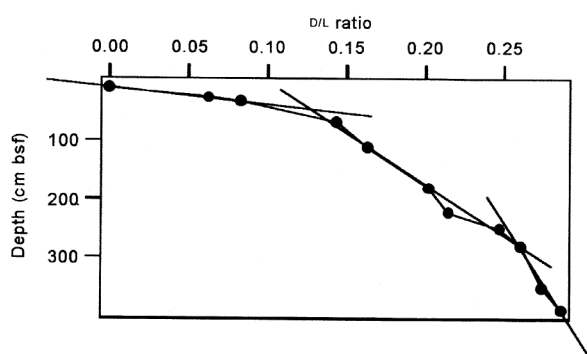


Figure 1. Aspartic acid D/L ratio vs depth. Each of the three regression lines shown represents a particular D/L horizon in which racemization constant is uniform.

acid racemizes faster¹⁴. In the monospecific *Pullenata obliquiloculata*, a planktonic foraminifer of the deep North Western Pacific sediments (water depth = 2831 m) also, the aspartic acid racemization constant fell from $0.94 \times 10^{-5} \text{ yr}^{-1}$ in the upper sediments to $0.099 \times 10^{-5} \text{ yr}^{-1}$ (i.e. by almost an order of magnitude) in the lower sediments of the core.

For the sediment samples lying in each of the three sediment horizons, amino acid D/L ages were computed (Table 1) from the respective racemization constant using eq. (1). The D/L ages thus computed range from ~1500 yr (26.5 cm) to ~50,000 yr (390.5 cm).

Assuming that the sedimentation rates have remained uniform at intervals, the rate calculated during the 4783–19,030 yr BP is 10.8 cm kyr^{-1} and only 5.8 cm kyr^{-1} earlier to that since 41 kyr BP. Still earlier, it was only 4.4 cm kyr^{-1} . Few reports of sedimentation rates are available for the western Bay of Bengal. At a water depth of 1200 m off Penna delta, Purnachandra Rao *et al.*¹⁵ suggested a Holocene sedimentation rate of 9.4 cm kyr^{-1} . In a core (2909 m water

depth) of the deep Andaman Sea, Ahmad *et al.*¹⁶ reported a LGP sedimentation rate of 8.3 cm kyr^{-1} , higher than the average rate of 5.3 cm kyr^{-1} for the core. The rates obtained from radiocarbon data and dates predicted from aminostratigraphy are in line with these reports.

- Hare, P. E. and Abelson, P. H., *Carnegie Inst. Washington, Yearb.*, 1967, **66**, 526–528.
- Brown, R. H., *Origins*, 1985, **12**, 8–25.
- Goodfriend, G. A., *Nature*, 1992, **357**, 399–401.
- Goodfriend, G. A., Brigham-Grette, J., and Miller, G. H., *Quat. Res.*, 1996, **45**, 176–187.
- Dittmar, T., Fitznar, H. P. and Kattner, G., *Geochim. Cosmochim. Acta*, 2001, **65**, 4103–4114.
- Goodfriend, G. A. and Stanley, D. J., *Mar. Geol.*, 1996, **129**, 271–283.
- Goodfriend, G. A., *Geochim. Cosmochim. Acta*, 1991, **55**, 293–302.
- Degens, E. T., Reuter, J. H. and Shaw, K. N. F., *Geochim. Cosmochim. Acta*, 1964, **28**, 45–66.
- Yadava, M. G. and Ramesh, R., *J. Sci. Ind. Res.*, 1999, **58**, 339–348.
- Stuiver, M. *et al.*, *Radiocarbon*, 1998, **40**, 1041–1084.

- Bard, E., Arnold, M., Hamelin, B., Tisnerat-Laborde, N. and Cabiocch, G., *Radiocarbon*, 1998, **40**, 1085–1092.
- Harada, N., Handa, N., Ito, M., Oba, T., and Matsumoto, E., *Org. Geochem.*, 1996, **24**, 921–930.
- Harada, N., Handa, N., Oba, T., Matsukawa, H., Kimoto, K. and Kusakabe, M., *J. Oceanogr.*, 1997, **53**, 1–7.
- Bada, J. L. and Man, E. H., *Earth Sci. Rev.*, 1980, **16**, 21–55.
- Purnachandra Rao, V., Rao, Ch. M., Mascarenhas, A., Mohana Rao, K., Reddy, N. P. C. and Das, H. C., *J. Geol. Soc. India*, 1992, **40**, 59–69.
- Ahmad, S. M., Patil, D. J., Rao, P. S., Nath, B. N., Rao, B. R. and Rajagopalan, G., *Proc. Indian Acad. Sci. (Earth Planet. Sci.)*, 2000, **109**, 153–156.

ACKNOWLEDGEMENTS. We thank the Department of Ocean Development, Govt. of India for financial assistance and shipboard facilities.

Received 9 December 2002; revised accepted 3 June 2003

NITTALA S. SARMA^{†, #}
M. S. RAMA KRISHNA[†]
SK. G. PASHA[†]
M. G. YADAVA*
K. MOHANA RAO**

[†]Marine Chemistry Laboratory,
School of Chemistry,
Andhra University,
Visakhapatnam 530 003, India
*Physical Research Laboratory,
Navrangpura,
Ahmedabad 380 009, India
**National Institute of Oceanography,
Regional Centre,
L.B. Colony,
Visakhapatnam 530 017, India
[#]For correspondence.
e-mail: nittalas@lycos.com