Chapter 2
MATERIALS AND METHODS

2.1 Introduction

In order to achieve the scientific objectives identified in chapter 1, three short and two long gravity cores were collected from the coastal region (off Goa) along the western continental margin of India and open ocean denitrifying zone of Arabian Sea respectively. The geochemical measurements were carried out using various analytical techniques and instruments. Apart from core collection, water sampling was also done at the time of core collection and during other field trips, to have the water column data. The detailed descriptions of sampling and material including methods are given below.

2.2 Collection of Sediment Cores

Two short gravity cores were collected in October 2002, during the first trial cruise of a multi-corer on board the CRV Sagar Sukti. One more gravity core was collected in September 2005 on board the CRV Sagar Sukti during the SaSu-98 cruise, using the two meter long gravity corer on board. The other two long gravity cores were collected in February 2002, during the 42nd cruise of M/V A.A. Siderenko, a vessel chartered by the then Department of Ocean Development, now Ministry of Earth Sciences (MoES). Gravity cores were collected by the author while the short coastal cores were collected by the biogeochemistry group from NIO. Details of the core location, water depth
of collection and length of the sediment cores raised, are given in Table 2.1. Location of all the cores is shown in figure 2.1.

Colour and lithology of sediments were noted immediately after the recovery of the cores on board. Visual examinations was done for identifying the presence of turbidites and sediment slumping. All the cores were sub-sampled on board into 1 cm thick slices, throughout for the short cores and 2 cm slices for the long cores, using thin transparent knife shape acrylic plate. The sediment samples were then sealed in plastic zipped bags and stored. At shore based laboratory, required amount of all the samples were oven dried at 60°C and preserved in pre-cleaned plastic vials until further analyses.

2.3 Various proxies used in this study

Multi-proxy studies are common in reconstructing environmental changes. Particularly, anthropogenic eutrophication and the onset of anoxia have been examined in various estuarine systems by number of researchers (e.g. Cornwell et al., 1996; Zimmerman and Canuel, 2000; Chmura et al., 2004) through the multi-proxy approach. To decipher the past changes in biological productivity, sub-surface denitrification, terrestrial inputs and bottom water redox conditions at seabed, various proxies viz. biogenic constituents, stable carbon and nitrogen isotopes, trace metals, and stable oxygen and carbon isotopes of forams were measured. A detailed analytical scheme followed for the systematic sediment sample analyses is shown in figure 2.2. Details of all the proxy analyses done in this study and the different
Table 2.1
Locations and details of sediment cores collected from the Arabian Sea.

<table>
<thead>
<tr>
<th>Cruise No.</th>
<th>Core No.</th>
<th>Latitude °N</th>
<th>Longitude °E</th>
<th>Water Depth (m)</th>
<th>Length of the Core (cm)</th>
<th>Collection date</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaSu - 98</td>
<td>CR-2</td>
<td>14°8.0'</td>
<td>74°2.0'</td>
<td>45</td>
<td>100</td>
<td>27/09/2005</td>
</tr>
<tr>
<td>SaSu Trial Cruise</td>
<td>SaSu-1</td>
<td>15°28.6'</td>
<td>73°27.5'</td>
<td>50</td>
<td>21</td>
<td>23/10/2002</td>
</tr>
<tr>
<td>SaSu Trial Cruise</td>
<td>SaSu-3B</td>
<td>15°28.4'</td>
<td>73°32.42'</td>
<td>35</td>
<td>43</td>
<td>23/10/2002</td>
</tr>
<tr>
<td>AAS - 42</td>
<td>AAS-42/15</td>
<td>17°13.0'</td>
<td>69°02.0'</td>
<td>2525</td>
<td>440</td>
<td>19/02/2002</td>
</tr>
<tr>
<td>AAS - 42</td>
<td>AAS-42/12A</td>
<td>15°4.0'</td>
<td>69°59.0'</td>
<td>2270</td>
<td>530</td>
<td>18/02/2002</td>
</tr>
</tbody>
</table>
Figure 2.1: Location of cores collected from the shallow and deep water OMZ region of Arabian Sea. The extent of OMZ is demarcated by the secondary nitrite maxima (0.5μM). Figure modified from Naqvi (1991).
Figure 2.2: Analytical scheme for chemical and isotopic analyses of sediment samples

Bulk Sediment Sample

Wet Sieved and dried for Foraminifers

Dried and homogenised

$^{14}$C-AMS
$^{13}$C-IRMS
$^{18}$O-IRMS

chemical composition

organic fraction

CaCO$_3$
Coulometer

C$_{org}$, N
CN analyzer

Major and trace elements
ICP-AES

$^{13}$C, $^{15}$N
(EA - Mass Spectrometer)
instruments used are discussed below, with the accuracies summarized in Table 2.4 in terms of precision.

2.3.1 \( ^{210}\text{Pb} \) and AMS \( ^{14}\text{C} \) dating

\( ^{210}\text{Pb} \) dating

Chronology of short cores (SaSu-1, SaSu-3B and CR-2) was constructed by the \( ^{210}\text{Pb} \) dating technique, measured via its grand daughter nuclide \( ^{210}\text{Po} \), at Physical Research Laboratory, Ahmedabad. About 2-3 g of ground dry sediment sample was leached with 8M HCl in presence of \( ^{209}\text{Po} \) spike. The solution was finally made in 50-60 mL of 0.6M HCl, and ascorbic acid was added to complex the \( \text{Fe}^{3+} \) ion present in the solution. The polonium (Po) isotopes were then autoplated onto a 1.5cm diameter silver disc suspended in solution. The plating was done for 3 hours by maintaining the temperature of the solution at \(-65^\circ\text{C}\). After plating, silver disc was rinsed with distilled water, dried and the activities of the Po isotopes (\( ^{209}\text{Po} \) and \( ^{210}\text{Po} \)) were measured for alpha counting using Si-surface barrier detectors coupled to a pulse height analyzer (Yadav et al., 1992; Sarin et al., 1992; Somayajulu et al., 1994, Sharma et al., 1994).

AMS \( ^{14}\text{C} \) dating

Chronology for the long gravity cores (AAS-42/15 and AAS-42/12A) was set up by \( ^{14}\text{C} \) dating of planktonic foraminiferal separates of \textit{Globigerinoide ruber} species (>250 \( \mu \text{m} \)) employing Accelerated Mass Spectrometer (AMS). The AMS measurements were performed at the
National Ocean Sciences AMS Facility (NOSAMS), Woods Hole, MA (USA). The samples were processed to derive the graphite form of carbon which was then compressed by the target press and inserted into the cathode (Cs) of the ion source. The sample was then accelerated and the carbon converted into a positive ion and then, the separated C-12 and C-13 ions were measured in Faraday Cups, where a ratio of their current was recorded. Simultaneously the C-14 ions were recorded in a gas ionization counter so that ratios of C-14 to C-13 and C-12 were recorded instantly. These raw data signals were then converted to radiocarbon ages using algorithms. Carrara Marble was used as a blank, while Oxalic Acid was used as the standard. Based on repeat standards analysis, the precision of the technique was found to be 5-7 per mil (McNichol and Aluwihare, 2007), (http://www.nosams.whoi.edu). A single sample (interval 97-99cm) from core CR-2 was also dated by AMS ¹⁴C for bulk organic matter, to ascertain the core age beyond the ²¹⁰Pb dating limits; at the Institute of Physics (IoP), Bhubhaneshwar.

2.3.2 Organic Carbon and Nitrogen analyses

All the cores (SaSu-1, SaSu-3B, CR-2, AAS-42/15, and AAS-42/12A) were analyzed for organic carbon and nitrogen proxies. Prior to analyses, sediment samples were treated with 1M HCl to remove the carbonates, washed thoroughly with de-ionized water, dried and homogenized except for those analyzed in Germany. Analyses of sediment samples for organic carbon (C_{org}) and nitrogen (N_{org}) were done on three different instruments as per their availability to carry out the analysis. SaSu-1 and SaSu-3B core samples were
measured by *Fisons NA1500 NC Elemental Analyzer* (Fisons Inc., Italy) at the Physical Research Laboratory (PRL), Ahmedabad. About 10mg of the dried, decalcified samples were taken for analysis. The samples were packed in aluminium cups and introduced in the Elemental Analyzer through an autosampler. The samples were dropped into the combustion tube containing silver cobaltous cobaltic oxide and granular chromium oxide maintained at 1050°C. In the combustion tube, the samples were combusted readily due to flash combustion in presence of high purity oxygen at ~1800°C. All the halides and sulphur gases were removed in the combustion column and the evolved CO₂ and NOₓ were then passed through a reduction column containing reduced Cu at 650 °C to yield finally purified CO₂ and N₂. The final pure CO₂ and N₂ were then separated in a GC column at 60 °C and measured by Thermal Conductivity Detector (Bhushan et al. (2001). A three point calibration sequence was made following linear-fit method using a Deer River Shale Standard as a reference material containing 2.53% carbon and 0.12% nitrogen (Sarin et al., 1997). The analytical precision for measurement of carbon and nitrogen was found to be ±1.0% and ±0.8% respectively based on repeat measurements of the sample and standard.

Organic carbon and nitrogen for AAS-42/15 core sediments were estimated by *NCS analyzer* (CE Instruments, model NCS-2500; at NIO, Goa). ~ 8mg of the samples were weighed in tin cups and introduced into the vertical quartz tube (filled with silver cobaltous cobaltic oxide and granular chromium oxide) and combusted completely at ~1000°C. The evolved CO₂ and NOₓ were then passed through a reduction column containing Cu filings,
where nitrogen oxides got reduced to molecular nitrogen while the CO₂ remained unchanged. The gas mixture was then passed through an anhydrome trap, for the absorption of moisture. The resulting purified gas mixture was eluted and separated by a Porapack column and subsequently detected by a TCD detector. The precision of the analysis was found to be better than ±1% using Urea (NH₂CONH₂) as a standard.

Analysis of sediment samples from core AAS-42/12A for organic carbon and nitrogen were done by a Carlo Erba NA 2100 elemental analyzer at Center for Tropical Marine Ecology - Bremen, Germany. Organic carbon (C₀rganic) and nitrogen was measured after removal of carbonate by adding 200 µL of 1M HCl and subsequent drying at 40 °C, by using ~10mg of sample taken into 9x10.5 mm silver cups. The decalcified samples then were combusted in an oxidation column at 1100 °C under the supply of oxygen. The gas mixture containing oxides of nitrogen, carbon, hydrogen and sulphur were then transported by Helium, to a column filled with silver to remove SO₂ if any. For reduction of nitrogen oxides (NOₓ) the gases were passed through another column filled with copper. A trap was used to remove water. N₂ and CO₂ were then separated on a gas chromatographic column and measured by a thermal conductivity detector (TCD). Accuracy was monitored by measuring standards after every five samples. Standards used were 'Leco 1009' with 0.034 ±0.01 weight %N and 0.85 ±0.09 weight %C or 'Leco 1012' with 0.13 ±0.04 weight %N and 1.30 ±0.04 weight %C. Depending on sample sizes and compositions the combustion tube was cleaned for every 5-15 measurements.
The mean accuracy for nitrogen measurements were ±0.01%, while carbon was measured with accuracy of ±0.22%.

### 2.3.3 CaCO₃ analysis

Carbonate Carbon was determined for SaSu-1, Sasu-3B, CR-2, AAS-42/15 and AAS-42/12A cores by Coulometry using UIC Coulometer, model CM 5014 (UIC Inc., USA). CO₂ was evolved from the samples through acidification, by treating nearly 30mg of homogenized sediment sample with 5 mL of 1M HCl at 50 °C. CO₂ free air (air was stripped off CO₂ by passing through 45% KOH scrubber solution) was used as a carrier gas for flushing CO₂ in the system. The CO₂ liberated was flushed by the carrier gas and dried by passing it through a post scrubber solution of 50% KI maintained at pH=3 (pH adjusted to 3 with 50% Sulphuric Acid) and a column of activated silica gel and drierite (moisture absorbing zeolite). The dried CO₂ was then passed through the coulometer titration cell (with Platinum-cathode and Silver–anode) containing monoethanolamine solution and a self indicator (thymol blue) (Bhushan et al., 2001). Pure and dried CaCO₃ (suprapure grade MERCK) was used as a standard for calibration. The precision of CaCO₃ analysis was found to be <2.5% based on repeated standard and sample analysis.

### 2.3.4 Stable isotope studies

Stable oxygen and carbon isotope compositions of calcareous marine organisms have been widely used to reconstruct the paleo environmental conditions. For oxygen, $^{18}$O/$^{16}$O ($\delta^{18}$O) ratio is used, while for carbon it was $^{13}$C/$^{12}$C and denoted as $\delta^{13}$C. They were measured using Mass
Spectrometers with dual inlet system. In the present study, isotope measurements of forams were done using the Micromass (Isoprime) Dual Inlet, Mass Spectrometer of GV Instruments at National Institute of Oceanography, Goa.

**Sample preparation**

Samples of the open ocean gravity cores (AAS-42/15 and AAS-42/12A) were chronicled prior to analyses of other proxies. Stable isotope analysis of oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) were used as proxies to find the chronology of the cores. Stable isotope studies were done on the foraminifera (forams) fossils from the sediment. For this, aliquots of sediment samples were wet sieved using a 63 micron mesh of diameter 12 inches, with tap water, dried and size fractionated for 250 and 125 µm using the respective sieves. The required species of planktonic and benthic forams were then hand picked using a simple stereo microscope with a resolution of 50X.

**Basic principle of Mass Spectrometers**

Mass spectrometers are the most effective and widely used means of measuring isotope abundances. In principle, a mass spectrometer deflects any moving charged particle under the influence of magnetic field and/or electric field. A mass spectrometer may be divided into four parts (Figure 2.3): (1) the inlet system, (2) the ion source and accelerator, (3) the mass analyzer, and (4) the ion detector (Hoefs, 1987). The whole system works under very high vacuum, which is essential for the stability of the ions produced and for their free flow.
**Schematic of ICP-AES**

**Figure 2.5:** Schematic diagram of an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) for elemental analysis.
The *inlet system* provides a facility for purification of gas (CO₂ in case of carbonates – forams) before feeding to the mass spectrometer. This is done through a series of trapping systems (moisture trap along with cold fingers 1 and 2) which removes the moisture contents and other unwanted gases produced from the sample materials during the reaction with acid (phosphoric).

The *ion source* is an electrically heated metal coil (Thorium–Th) that gives off electrons. The atoms/molecules from the sample are bombarded with this stream of electrons (perpendicular to each other), during which some of the collisions are energetic enough to knock one or more electrons out of the atoms/molecules to make positive ions. These molecular positive ions are then repelled away from the very positive ionization chamber and accelerated into a finely focused beam.

The *mass analyzer* separates the ion beams emerging from the ion source based on their mass/charge ratio (mass/charge ratio is given the symbol m/z or sometimes m/e). This is done through a strong magnetic field. As the ion beam passes through the magnetic field, the ions get deflected into circular paths based on their masses (i.e. m/z ratio) as the charge on all ions is preferably assumed to be 1 and it requires a lot more energy to knock off the second electron from an already positive ion. The radii of the circular path are proportional to the square root of m/z ratio.

The ion detector (Faraday cups) collects the separated ion beam (coming from the analyzer) and converts it into electrical impulses, which are then fed into an amplifier. In case of oxygen and carbon isotope
measurements, there are separate Faraday cups to detect the different masses of ions simultaneously.

2.3.4.1 Stable Oxygen ($\delta^{18}$O) and Carbon ($\delta^{13}$C) Isotope analysis

Stable isotope measurements of both oxygen and carbon were performed on surface dwelling planktonic foraminifers - *Globigerinoide ruber* and benthic foraminifer - *Cibicidoides wuellerstorfi*, for both the open ocean gravity cores. For planktonic foraminifers, tests from >250 µm fraction were used for analysis to avoid the size fraction effect (Duplessy et al., 1981). Prior to analysis, the specimens were ultra sonicated for 5-10 seconds (taking care not to break the tests) to remove any fine material attached to the tests. The tests (mounted on special slides with holes) were then dried and preserved in a desiccator until analysis was done. The isotope (carbonates) measurements were carried out on the GV- *Micromass Spectrometer* coupled with an automated CO$_2$ preparation system (Gilson - multicarb). The isotopic composition of carbonate was measured on the CO$_2$ gas evolved by the treatment of foraminiferal shells with 102.6% pure, specific gravity 1.913g/cm$^3$ ortho-phosphoric acid at a constant temperature of 90 ºC through an auto-sampler. The ortho-phosphoric acid was purified by distillation in the laboratory from analytical grade 88% with specific gravity 1.75g/cm$^3$. The standard CO$_2$ gas was then calibrated against the international carbonate standard – Pee Dee Belemnite (PDB), using National Bureau of Standards 19 (NBS-19). The denoted unit of isotope ratio measurements is the delta value ($\delta$). The $\delta$-value is defined in the same way as mentioned in equation (1) as:

\[ \delta = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \]
\[ \delta^{18}O = \left\{ \frac{\left( ^{18}O/^{16}O \right)_{\text{sample}}}{\left( ^{18}O/^{16}O \right)_{\text{standard}}} - 1 \right\} \times 1000 \]  

and

\[ \delta^{13}C = \left\{ \frac{\left( ^{13}C/^{12}C \right)_{\text{sample}}}{\left( ^{13}C/^{12}C \right)_{\text{standard}}} - 1 \right\} \times 1000 \]

The \( \delta \) notation represents per mil (‰) deviations from the isotopic standard

PDB, prepared from the rostrum of the belemnite - Belemnitella Americana

from the Cretaceous Pee Dee formation of South Carolina. The PDB standard

is defined as 0 per mil for carbon and oxygen. The isotopic composition of

water was reported as per mil deviations of the sample, from Standard Mean

Ocean Water (SMOW); a hypothetical water close to average ocean water

(Craig, 1957). Since the supply of PDB is exhausted, calibrations are normally
done through the analysis of NBS samples. The NBS 19 is a homogenized

standard of Jurassic Solnhofen limestone form Southern Germany. The

standard deviation, (i.e. the long term measurement precision) based on

replicate analyses of internal laboratory standards and NBS19 for \( \delta^{18}O \) were

better than \( \pm 0.06\text{‰} \) and that for \( \delta^{13}C \) was better than \( \pm 0.04\text{‰} \). The mean

sample size was 3-5 shells for \( \delta^{18}O \) measurements while for \( \delta^{13}C \)

measurements an approximate sample weight of 20-60\( \mu \)g was taken.

2.3.4.2 Analyses of \( \delta^{13}C \) and \( \delta^{15}N \) in sedimentary organic matter

Measurements of \( \delta^{13}C_{\text{org}} \) and \( \delta^{15}N \) were done in the organic fraction of

the sediments for all the five cores (SaSu-1, SaSu-3B, CR-2, AAS-42/15, AAS-42/12A) using an isotope ratio mass spectrometer of Thermo Finnigan

Delta plus, at the Center for Tropical Marine Ecology - Bremen, Germany. The
mass spectrometer was coupled with the \textit{Flash EA 1112 elemental analyzer} in a continuous flow mode. Required amount of samples were weighed in silver cups and combusted. The gas mixture ($\text{N}_2$ and $\text{CO}_2$) was separated in a Flash EA 1112 elemental analyzer (same procedure as in the NA 2100 elemental analyzer mentioned in section 2.3.2). Carbon dioxide ($\text{CO}_2$) and Nitrogen ($\text{N}_2$) were ionized and transferred into a magnetic field, where they were separated by virtue of their different mass/charge ratio. The split ion beams were detected and the results were expressed as $\delta$ values in $\%\text{o}$, as deviation from composition of atmospheric nitrogen and V-PDB standard for carbon, $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ were calculated as follows:

$$
\delta^{13}\text{C}_{\text{org}} \text{ and } \delta^{15}\text{N} = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000
$$

Where $R = ^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$.

Calibration for $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ contents was achieved using several international standards throughout the analyses. A sample from Brazil was frequently used as a reference and this standard was measured after every 7 samples (Pedersen et al., 1991, Ganeshram et al., 1995, Higginson et al., 2004). The standard deviation for $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ was found to be $\pm 0.11$ and $\pm 0.17\%\text{o}$ respectively.

\textbf{2.3.5 Elemental analysis of major and trace elements}

Major and trace elements were analyzed for cores SaSu-1, Sasu-3B, CR-2, AAS-42/15 and AAS-42/12A using the Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) technique. The spectrometry method required the sample to be introduced in solution form. For this, the
samples were digested using an appropriate acid mixture employing the closed vessel technique.

2.3.5.1 Sample preparation with MARS

The choice of digestion method depends on the requirement of extent of dissolution of the sample, quantities of acids, total digestion taken, and minimal analytical errors. Microwave-acid digestion utilizes lesser amounts of acids and is faster with minimal contamination than open vessel digestion. Also an important factor to be considered is the nature of the geological material (Totland et al., 1992). Keeping all these aspects in mind, microwave digestion technique was adopted for the dissolution of sediment samples. Schematic diagram of the digestion procedure is shown in figure 2.4. Dried, homogenized, sediment samples weighing ~500mg were taken in clean Teflon liners. To the liners then a mixture of concentrated, ultra pure (metal free) acids in the proportion of 5:3:2 mL of HNO₃: HF: HCl respectively was added (Morford and Emerson 1999; Crusius et al., 1996). However the acid combination was finalized after a series of laboratory experiments with different combination of all the acids (Rosenthal et al., 1995b; Reichart et al., 1998; Warnken et al., 2001). The Teflon vessels were then screw capped (fitted with rupture membrane for safety purpose) and placed in Microwave Accelerated Reaction System (MARS; model MARS5, CEM, USA). A two-step procedure as given in table 2.2 was followed to ensure complete digestion of the sediment samples. The final volume was made to 50 mL. For analysis of major elements, samples were accordingly diluted and measured.
Table 2.2
Two step procedure for digestion of dried, homogenized sediment samples for elemental analysis on ICP-AES.

**Step 1: Acid mixture (HNO₃, HF, HCl)**

<table>
<thead>
<tr>
<th>Microwave Power (watts)</th>
<th>No. of Vessels</th>
<th>Power (%)</th>
<th>Ramp Time (min)</th>
<th>Control Pressure (psi)</th>
<th>Control Temp. (°C)</th>
<th>Hold Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200*</td>
<td>12</td>
<td>90</td>
<td>30.0</td>
<td>800</td>
<td>210</td>
<td>30.0</td>
</tr>
</tbody>
</table>

*Microwave power is decided based on the number of vessels used for digestion as 1200W for 12; 600W for 6 and 300W for 3 vessels.

**Step 2: Boric Acid (15-20mL for each sample)**

| 1200* | 12 | 85 | 20.0 | 800 | 180 | 30.0 |

*Microwave power is decided based on the number of vessels used for digestion as 1200W for 12; 600W for 6 and 300W for 3 vessels.
~500mg dried, homogenised sediment sample

Treated with conc. HNO₃+HF+HCl in MARS at 210°C

Residue treated with sat. Boric acid in MARS at 180°C

Final solution made in 0.05M HNO₃

Analyzed with ICP-AES

**Figure 2.4:** Schematic diagram of digestion procedure on MARS-5 for elemental analysis.
on ICP-AES. Standards (USGS) were also treated the same way as the samples.

2.3.5.2 Measurements on ICP-AES

The digested samples were measured for major and trace elements using the Liberty-Series II (Sequential mode) ICP-AES of Varian make. The major elements measured were Aluminium (Al), Magnesium (Mg), Iron (Fe), Strontium (Sr), Titanium (Ti) and Phosphorus (P) and among the trace elements, Manganese (Mn), Molybdenum (Mo), Vanadium (V), Cobalt (Co), Nickel (Ni), Copper (Cu), Barium (Ba) and Chromium (Cr). A schematic of the ICP-AES is shown in figure 2.5. The sample gets introduced into the nebulizer with the help of pneumatic peristaltic pump, where it is converted into an aerosol with the help of an inert Argon gas flowing perpendicular to the sample flow. The fine mist is then carried to the plasma flame (6,000-10,000°C) where the sample gets atomized, ionized and excited. The argon plasma works at very high radio frequency and in this instrument it is operated at 36MHz. The light emitted from the plasma is focused onto the holographic grating which resolves the light into its individual wavelengths specific for each element. The light signal is then taken up by the photomultiplier tube (PMT) detector, which generates a milli-volt signal proportional to incident light signal. The electrical signal then gets converted in terms of concentration with the help of a capacitor and a digital voltammeter. All the specifications and operating conditions of the ICP-AES are given in table 2.3.

Calibration of the spectrometer was achieved by means of multi-
### Table 2.3
Specifications and operating conditions of ICP-AES.

<table>
<thead>
<tr>
<th>ICP Spectrometer</th>
<th>Liberty Series II, Varian (sequential mode)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>1.2 KW</td>
</tr>
<tr>
<td>Plasma gas flow</td>
<td>12.0 L.min⁻¹</td>
</tr>
<tr>
<td>Auxiliary gas flow</td>
<td>1.5 L.min⁻¹</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>V-groove</td>
</tr>
<tr>
<td>Sample uptake rate</td>
<td>1 mL.min⁻¹</td>
</tr>
<tr>
<td>PMT voltage</td>
<td>700-800volts</td>
</tr>
<tr>
<td>Signal integration</td>
<td>3 seconds</td>
</tr>
<tr>
<td>Grating order</td>
<td>Default</td>
</tr>
<tr>
<td>Filter position</td>
<td>Default</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.006 nm for Cd line at 228.8 nm</td>
</tr>
<tr>
<td>Detection limit</td>
<td>$3 \sigma \mu g L^{-1}$</td>
</tr>
</tbody>
</table>
Figure 2.3: Schematic diagram of an isotope ratio mass spectrometer (IR-MS) for stable isotope measurements. The dual inlet (DI) section is modified from Hoefs, (1987) where 'P' denotes pumping system and 'V' a variable volume.
element standards in 0.5 M HNO₃ of varying concentrations at ppb and ppm levels as per the element requirement during analysis. Several USGS rock standards like MAG-1 (marine sediment), SCo-1(Cody shale/silty marine shale), and BIR-1(Icelandic Basalt) were analyzed to check accuracy and precision of the elemental analysis of sediment samples. Among all the standards, MAG-1 was analyzed extensively in this work as it closely matched the matrix of the sediment samples. Precision involved for the elements measured were 1-5%.

2.4 Water sampling and analysis

During the core collection from the deep sea, water samples were collected from selected (mostly standard) depths covering the entire water column (down to bottom) using either Go-flo or Niskin samplers (5/10 -litre capacity) mounted on rosette fitted to Sea-bird CTD (conductivity-temperature-depth, No. SBE 9/11). Temperature and conductivity sensors in the CTD units allowed continuous profiling of these properties. The salinity data derived from the in-situ sensor was calibrated through analysis of discrete samples in the laboratory. Temperature recorded by probe was occasionally verified by using reversible thermometers. While for the coastal sample collection, a portable CTD was generally used. Again the temperature profiling data from the CTD was checked with reversible thermometer readings and check for salinity was done by analyzing samples with an Autosal Salinometer (model 8400).
Once the CTD sampler was brought on the deck, samples were collected following a particular fashion. Firstly, salinity samples were collected in glass bottles after a thorough rinsing (three times) and filled up to the shoulder. The neck of the bottle was dried with a tissue paper so as to avoid salt deposition. The bottles were then capped tightly and kept in a temperature-controlled room until analysis. Salinity values are expressed as Practical Salinity Units (psu, Fofonoff, 1983).

The samples then collected for other chemical analyses, were first sampled for dissolved gases (O_2, H_2S, N_2O) in specialized glass bottles and then for nutrients in teflon plastic bottles. While sampling for dissolved gases, utmost care was taken to avoid any atmospheric contamination. Chemical analyses of discretely-collected water samples for O_2, hydrogen sulphide (H_2S), and nutrients (NO_3^−, NO_2^− and NH_4^+) were performed on board ships, whereas those samples collected during the coastal field trips were done in the shore laboratory. N_2O samples were preserved with HgCl_2 and analyzed either on board ship or in the shore laboratory.

2.4.1 Analyses of dissolved gases

2.4.1.1 Dissolved oxygen

Dissolved oxygen was estimated by the Winkler titration method as modified by Carpenter (1965). The principle of the method is as follows:

The dissolved oxygen in seawater was made to oxidize Mn (II) to Mn (III) to Mn (IV), under strongly alkaline medium. In the presence of excess iodide, reduction of Mn (III) and Mn (IV) liberates iodine upon acidification (either
H₂SO₄ or HCl). The iodine released was titrated against standard sodium thiosulphate solution using starch as the indicator. The amount of O₂ was calculated from sodium thiosulphate consumed.

2.4.1.2 Hydrogen Sulphide

Hydrogen sulphide was estimated spectrophotometrically by methylene blue method by Fonselius (1962). The method is based on dimethyl-p-phenylene diamine reaction in acidic medium with ferric chloride to form an indammonium salt (Bindshedler's green), an intermediate. The product then combines with hydrogen sulphide yielding a thiazine dye (methylene blue). This compound's maximal absorbance occurs at 670 nm and was measured at this particular wavelength.

2.4.1.3 Nitrous Oxide

The estimation of N₂O was carried out by multiple phase analysis using the equilibriation technique of McAuliff (1971). An aliquot of 25 mL of seawater sample was taken in 100mL air-tight syringe that was previously flushed with helium gas to avoid atmospheric contamination. An amount of 25 mL of helium gas was injected into the syringe. During the transfer of both, the water sample and helium gas utmost care was taken to avoid contact with atmosphere. The sample was equilibrated for 5 minutes by vigorous shaking on a mechanical shaker. The N₂O released from water into the headspace was dried by passing over a moisture trap (10cm long, 1cm wide). The sampled gas was then introduced in to the GC (via a 6-port valve) containing
a stainless steel column packed with Chromosorb (80/100 mesh) and maintained at 80 °C. The separated N₂O gas was then detected using Electron Capture Detector (ECD). The extraction was repeated twice, more on the same aliquot. The detector output was read in terms of peak area, by a data station. The precision of the method was ~ 4%. Standards were run at the beginning and end of each set of samples to check the drift in the instrumental conditions and response. Air was used as a working standard. Standardization was against a standard mixture of 510 ppb N₂O in nitrogen (Gas standards, Alltech Associated Inc, IL. USA). Calculations for getting the concentration of nitrous oxide were done by plotting the log of peak area of each extraction against the extraction number. The slope (z) and intercept (I) of each sample was computed and the initial concentration of nitrous oxide (N₂O) was then obtained from the following equation,

\[ [N_2O] = \frac{I}{z-1} \]

The concentration unit used for Nitrous Oxide measurements was part per million (ppm).

2.4.2 Nutrient analyses

Nutrient analyses were done using an automated SKALAR segmented flow analyzer (Model 5100-1), following the principles discussed in Grasshoff et al. (1983). The primary standards for analysis were prepared in bulk and stored aseptically in ampoules to maintain uniformity.
2.4.2.1 Nitrite and Nitrate

The estimation of nitrite in seawater was based on its reaction with an aromatic amine that led to the formation of a diazonium compound, which on coupling with a secondary aromatic amine forms an azo dye (Bendschneider and Robinson, 1952). The absorbance of the pink colored azo dye was measured at 540 nm.

The nitrate in seawater was determined based on the reduction of nitrate to nitrite in a reductor column filled with copper-amalgamated cadmium granules following which, nitrite was determined via the formation of an azo dye (Grasshoff, 1969). The reduction conditions were maintained using ammonia-ammonium chloride buffer in such a way that nitrate was quantitatively converted to nitrite but not further. The principal reaction that takes place is

\[ \text{NO}_3^- + \text{Me}_3 + 2\text{H}^+ \rightarrow \text{NO}_2^- + \text{Me}^{++} + \text{H}_2\text{O} \]

2.4.2.2 Ammonia (\(\text{NH}_4^++\text{NH}_3\))

Ammonia estimation was based on the improved method given by Koroleff (1970). Ammonia dissolved in seawater reacts with hypochlorite under moderately alkaline conditions forming monochloramine, which in the presence of sodium nitroprusside (as catalyst), phenol and excess hypochlorite forms indophenol blue. The ratio of phenol:active chlorine must be constant and should be close to 25 w/v which otherwise will affect (bleach) the colour intensity. The blue colour of the indophenol was then measured at a wavelength of 630 nm.
2.4.2.3 Phosphate

Inorganic phosphate was estimated using the method given by Koroleff (1983). Phosphate ions in seawater were made to react with acidified molybdate to yield a phosphomolybdate complex, which was then reduced to highly coloured blue compound by ascorbic acid. The absorbance of formed phosphomolybdenum blue was measured at 880 nm. To avoid the interferences from silicate, the pH of the final reaction was less than 1 and the ratio of sulphuric acid to molybdate was maintained between 2 and 3.
Table 2.4
Summary of precision for the analytical instruments employed during analyses.

<table>
<thead>
<tr>
<th>Instrument Name</th>
<th>Proxy analyzed</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scintillation Counter (RPL, Ahmedabad)</td>
<td>$^{210}$Pb</td>
<td>$\pm 5%$</td>
</tr>
<tr>
<td>Accelerator Mass Spectrometer (Woods Hole, USA)</td>
<td>$^{14}$C</td>
<td>$5 - 7%$</td>
</tr>
<tr>
<td>NC Elemental Analyzer (PRL, Ahmedabad)</td>
<td>$C_{org}$</td>
<td>$\pm 1.0%$</td>
</tr>
<tr>
<td></td>
<td>$N_{org}$</td>
<td>$\pm 0.8%$</td>
</tr>
<tr>
<td>NCS analyzer (NIO, Goa)</td>
<td>$C_{org}$</td>
<td>$\pm 0.9%$</td>
</tr>
<tr>
<td></td>
<td>$N_{org}$</td>
<td>$\pm 0.77%$</td>
</tr>
<tr>
<td>Elemental analyzer (Bremen, Germany)</td>
<td>$C_{org}$</td>
<td>$\pm 0.22%$</td>
</tr>
<tr>
<td></td>
<td>$N_{org}$</td>
<td>$\pm 0.01%$</td>
</tr>
<tr>
<td>Coulometer (NIO, Goa)</td>
<td>CaCO$_3$</td>
<td>$\pm 1.0%$</td>
</tr>
<tr>
<td>IRMS (NIO, Goa)</td>
<td>$\delta^{18}$O</td>
<td>$\pm 0.06%$</td>
</tr>
<tr>
<td></td>
<td>$\delta^{13}$C</td>
<td>$\pm 0.04%$</td>
</tr>
<tr>
<td>EA - MS (Bremen, Germany)</td>
<td>$\delta^{13}C_{org}$</td>
<td>$\pm 0.11%$</td>
</tr>
<tr>
<td></td>
<td>$\delta^{15}N_{org}$</td>
<td>$\pm 0.17%$</td>
</tr>
<tr>
<td>ICP-AES (NIO, Goa)</td>
<td>Fe &amp; V, Mn &amp; Mo, Co &amp; Cd</td>
<td>$&lt;1%, &lt;1.5%$, $&lt;3%$</td>
</tr>
<tr>
<td>GC (NIO, Goa)</td>
<td>N$_2$O</td>
<td>$&lt; 4%$</td>
</tr>
<tr>
<td>Autoanalyzer (NIO, Goa)</td>
<td>NO$_2^-$, NO$_3^-$, NH$_4^+$, PO$_4^{3-}$</td>
<td>$\pm 0.006, 0.06, 0.06, 0.003 \mu$M</td>
</tr>
</tbody>
</table>