

# **Environmental sampling methods and analysis**

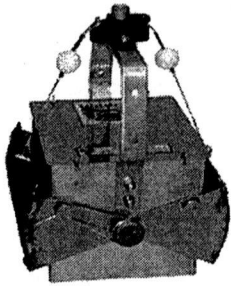
**By**

**Mrs. Buddhika Perera  
Mr. Namal Athukorala  
Dr. Suresh P. Benjamin**

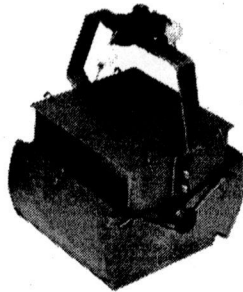
**Ecology and Environmental Biology Project  
Institute of Fundamental Studies (IFS)  
Kandy**

# Examples of some Limnological Equipment

## Sapling Equipment

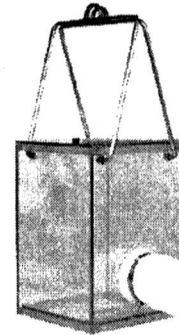


Open

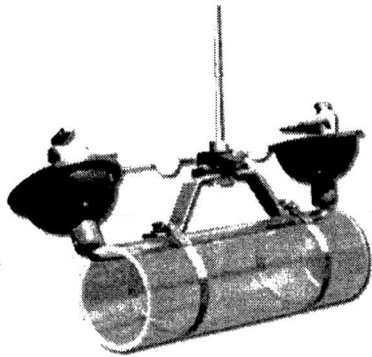


Closed

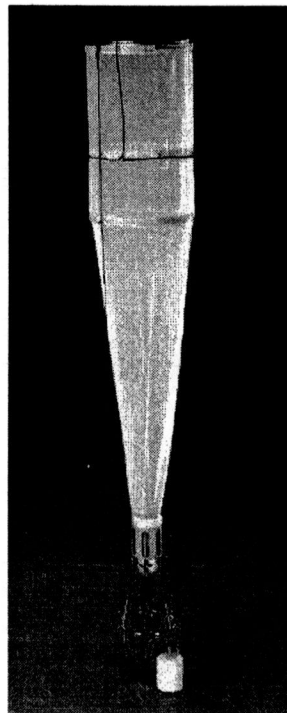
**Ekman Grab**



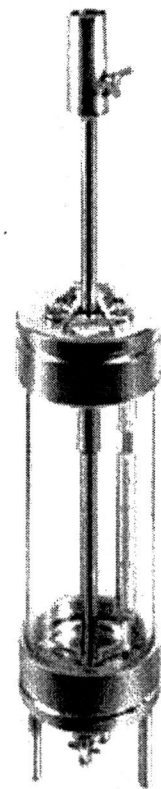
**Schindler Trap**



**Van Dorn Sampler**

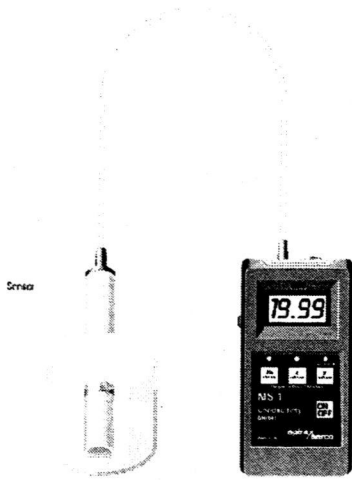


**Plankton net**



**Ruttner sampler**

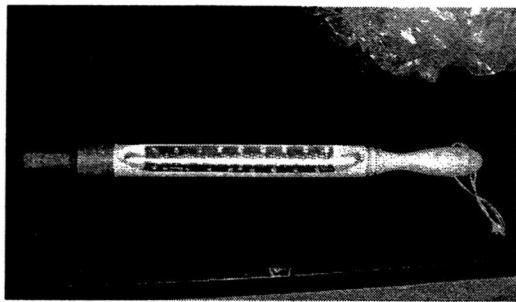
## Analyzing Equipment



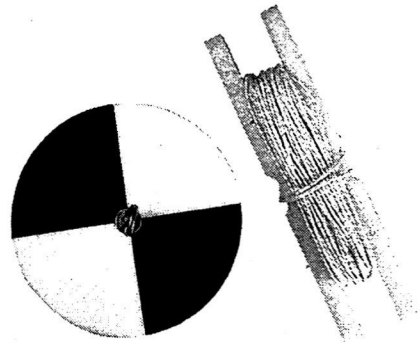
**Conductivity meter**



**pH meter**



**Thermometer**



**Secchi disk**



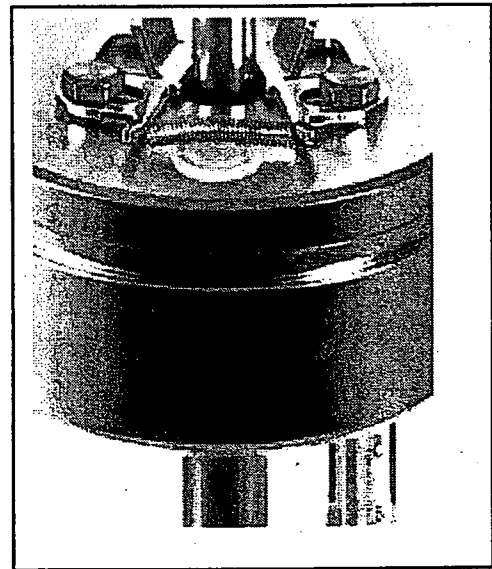
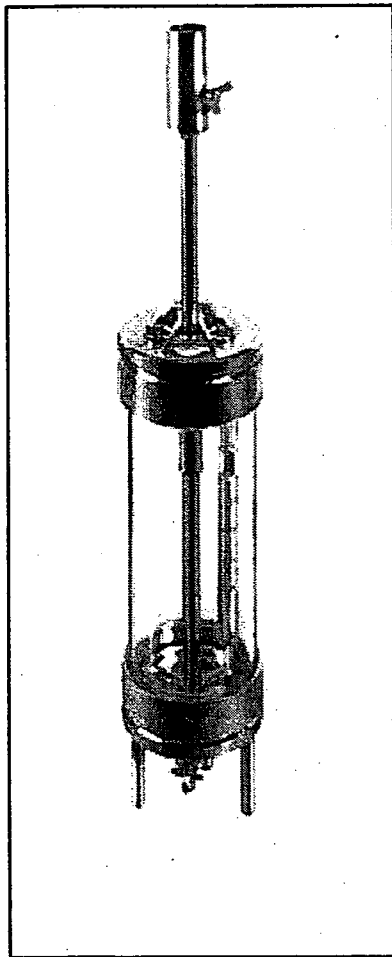
**Spectrophotometer**

## How to use ???

### Standard Water Sampler

The sampler, open, is lowered by rope into the water body. When it reaches the desired depth, a messenger is let down on the rope. Upon striking the standard sampler, it releases the closing mechanism and the lids of the sampling tube closes. In some versions of the water samplers, a second rope is used to close the sampling bottle.

The advantage is that no mixing of air with the sample will occur. The major disadvantage is that the bottle is in contact with water at all the depths through which the sampler travels on its way to the desired depth.



**Figure : Standard water sampler (Ruttner water sampler)**

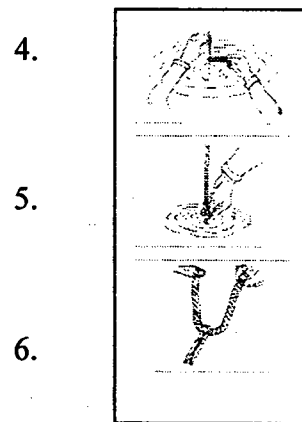
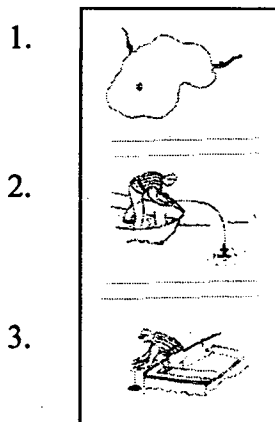
## Secchi Disk



A Secchi disk is an 8-inch (20 cm) disk with alternating black and white quadrants. It is lowered into the water of a lake until it can be no longer seen by the observer. This depth of disappearance, called the Secchi depth, is a measure of the *transparency* of the water. Transparency can be affected by the color of the water, algae, and suspended sediments. Transparency decreases as color, suspended sediments, or algal abundance increases. Water is often stained yellow or brown by decaying plant matter. In bogs and some lakes the brown stain can make the water the color of strong tea. Algae are small, green aquatic plants whose abundance is related to the amount of plant nutrients, especially phosphorus and nitrogen. Transparency can therefore be affected by the amount of plant nutrients coming into the lake from sources such as sewage treatment plants, septic tanks, and lawn and agricultural fertilizer.

Transparency is an indicator of the impact of human activity on the land surrounding the lake. If transparency is measured through the season and from year to year, trends in transparency may be observed. Transparency can serve as an early-warning that activities on the land are having an effect on a lake.

1. Go to your normal sampling site.
2. Anchor the boat to prevent drifting. Be careful not to disturb the sediments on the bottom when anchoring since this could cloud the water and interfere with the Secchi disk reading, especially in shallow lakes.
3. Lower the Secchi disk straight down into the water until the disk just disappears from sight. Mark the rope at the water level with a clothespin.
4. Slowly raise the disk up until it reappears. Mark the rope at the water level with your fingers or with the other clothespin.
5. To find the Secchi depth, grasp both clothespins in one hand and find the center of the loop of rope.
6. Move one clothespin to that point and remove the other. This point is one-half the distance between the point of disappearance of the disk and the point where it reappeared. Measure the distance from this point to the surface using a yardstick. Record the Secchi depth on your data sheet to the nearest cm.



## Introduction to Plankton

The term "plankton" comes from the Greek word "drifter" or "wanderer" and plankton includes all living organisms, both plant and animal, that drifts about passively in water either in the ocean or in fresh water bodies. Most of these organisms are microscopic, although some forms are visible to the naked eye. Also, many are capable of swimming in water, but because they are generally small in size, their movement in water is relatively slow and the distribution is determined by the water current.

Plankton can be categorized to two broad groups, phytoplankton the drifting plants and zooplankton the drifting animals. Phytoplankton is made up of usually, the algae, diatoms and desmids and they produce their own food by trapping the energy from the sun and carrying out photosynthesis. Zooplanktons, on the other hand, are the secondary consumers of the food chain. Copepods, cladocerans, ostracods & rotifers are the prominent members of the zooplankton in tropical freshwaters. Larval stages of most of the zooplanktons are present in the freshwaters and these provide an easy means of dispersal of the zooplankton before they become adults.

Several terms are used to describe the plankton. For example, the terms holoplankton and meroplankton respectively, are used to describe organisms that spent either their whole lives or part of their life histories. Drifting passively in the water. Many of the plankton organisms are microscopic, but there is a great variation in size among these. According to the different sizes, the plankton organisms are described as follows:

Size range of planktonic organisms :

0.2 – 2.0 m	- megaplankton ( eg :- jelly fish)
2.0 – 20 cm	- macroplankton ( eg :- some shrimps)
0.2 – 20 mm	- mesoplankton ( eg :- copepods)
20 – 200 $\mu$ m	- microplankton ( eg :- protozoa)
2.0 – 20 $\mu$ m	- nanoplankton ( eg :- autotrophic flagellates)
< 2.0 $\mu$ m	- picoplankton ( eg :- bacteria)

Both zooplankton and phytoplankton are in constant use as indicators of limnological conditions of fresh water bodies such as lakes and reservoirs.

## Sampling of Plankton

### a. Qualitative Sampling:

#### Collection and Preservation

In sampling the plankton organisms, one has to take into account their size and the distribution pattern. Most of the plankton organisms are microscopic and therefore, special nets have to be used for obtaining samples of these from their respective habitats. The mesh size of the plankton net is important in the exercise and selection of it depends on the objectives of the research project that you are interested in.

For sampling phytoplankton organisms the mesh size of the net should be  $\leq 50\mu$ . For collecting zooplankton sample a net with the mesh size ranging from  $50\mu$  to  $200\mu$  is used.

Lugol's solution could be used for preserving the phytoplankton samples. (The Lugol's solution makes the phytoplankton to sediment in the preserving vessel and at this stage the organisms could be examined using an inverted microscope). The sample should be preserved in 5% formaldehyde solution for longer – period storage.

### b. Quantitative Sampling:

#### Plankton Counting – Sedgwick Rafter Cell Method

A Sedgwick Rafter Cell (S-R cell) (Figure: 1) could be used to count the organisms present in the sample. The S-R cell has a rectangular cavity placed at the center of a slide. The cavity is 50 mm long, 20 mm wide and 1 mm deep, so as to hold exactly 1 ml of water sample. After transferring the sample it is covered by a slightly thicker cover slip before the counts are made under the stereo-microscope. The S-R cell is moved slowly under the stereo-microscope diagonally up and down or horizontally to make the plankton counts.

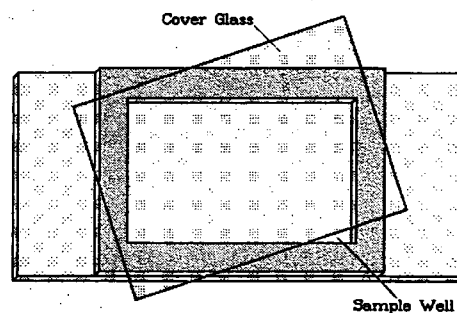
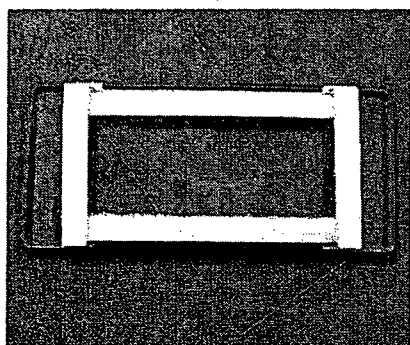
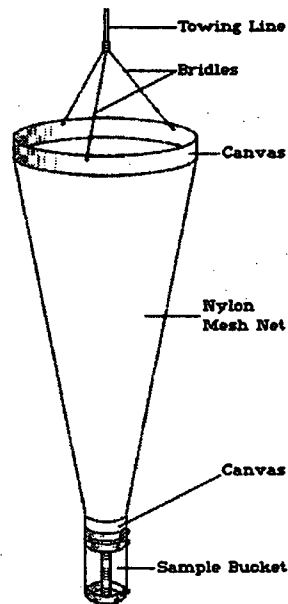


Figure : 1

A quantitative plankton sample could be obtained by filtering a known volume of water (10 ml) through a plankton net. Alternately, a plankton net (Figure:2) could be dragged along from a boat at a slow speed to get a quantitative sample from the surface waters of a lake.



**Figure: 2**

The sample, thus obtained is centrifuged and the supernatant water is decanted as much as possible. The remaining residue is made up to 10 ml with distilled water. Pipette out 1 ml of this sample and pour it carefully into the S-R cell and cover it up with a cover slip taking care not to entrap any air bubbles.

Move the S-R cell diagonally under a stereomicroscope and count the number of organisms (different taxa separately) present in 50 squares. Repeat the same exercise 3 times to get the average value.

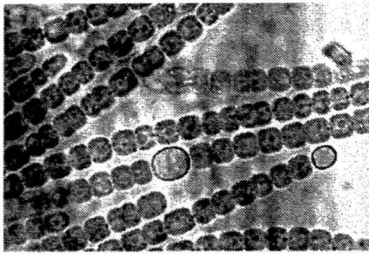
**Calculation :**

Let the volume of water filtered (P) through the plankton net = 10 l  
 Centrifuge the filtrate to Y ml (10) and examine y ml (3) from it.  
 Count the organisms in S number of squares (50) in the S-R cell  
 Let the total number of specific organism counted be M (125)  
 Then the abundance (N) of the specific plankton organism in the sample would be,

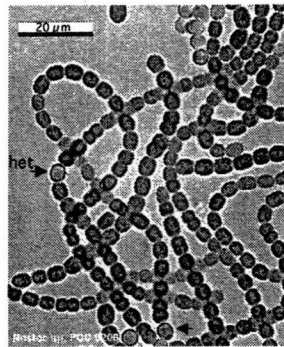
$$N = 125 \times (1000/50) \times (10/3) \times (1/10) = 833 \text{ per liter.}$$

# Some common fresh water plankton

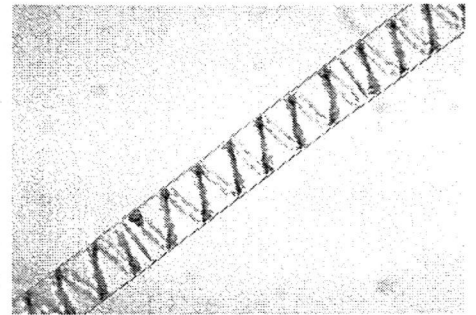
## Phytoplankton



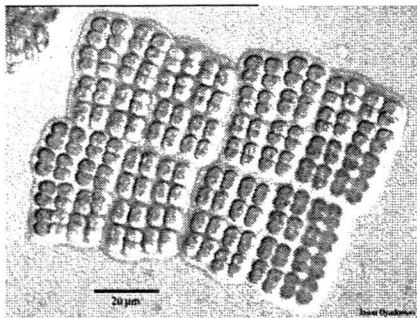
*Anabaena* sp.



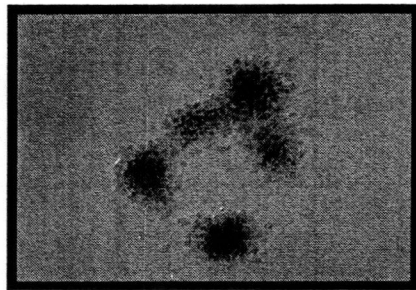
*Nostoc* sp.



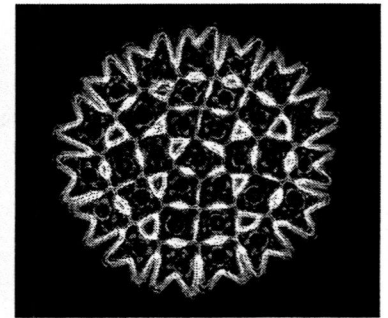
*Spirogyra* sp.



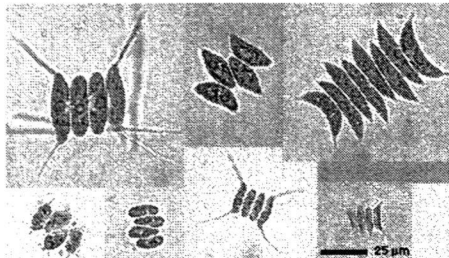
*Merismopedia* sp.



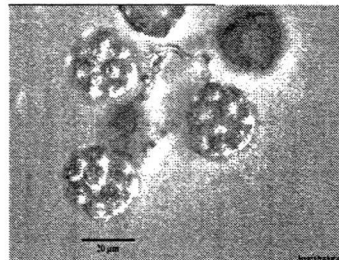
*Microcystis* sp.



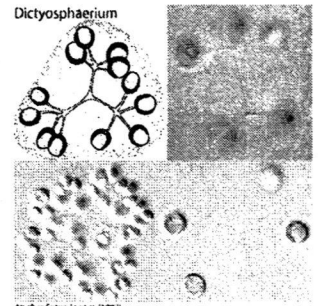
*Pediastrum* sp.



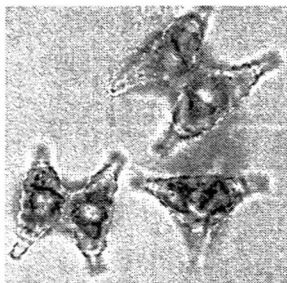
*Scenedesmus* sp.



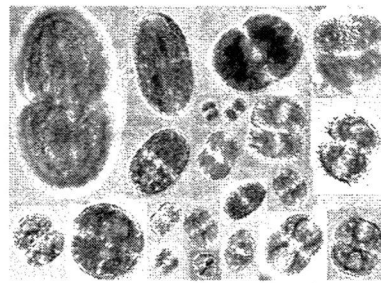
*Coelastrum* sp.



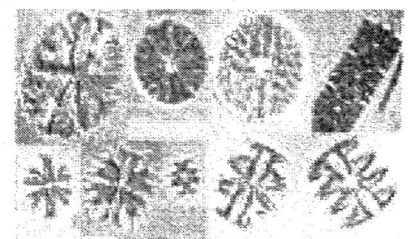
*Dictyosphaerium* sp.



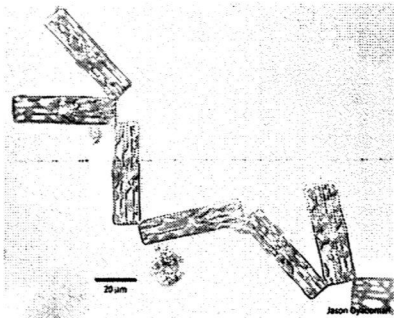
*Staurastrum* sp.



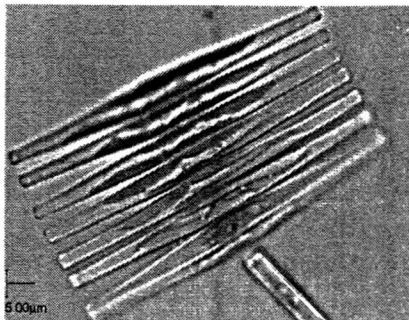
*Cosmarium* sp.



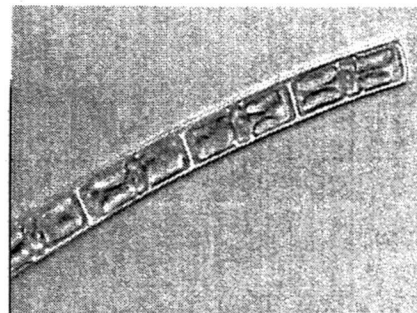
*Micrasterias* sp.



*Tabellaria* sp.



*Fragillaria* sp.

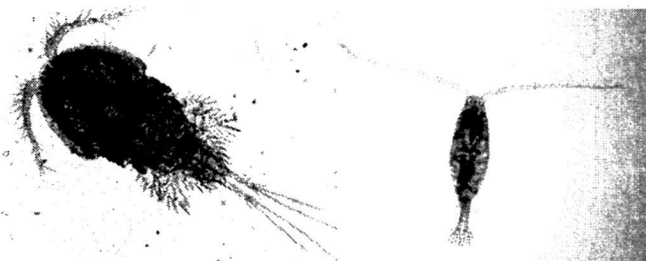


*Aulacosiera* sp.

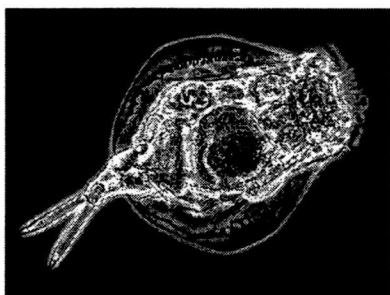
## Zooplankton



Cladocera



Copepods

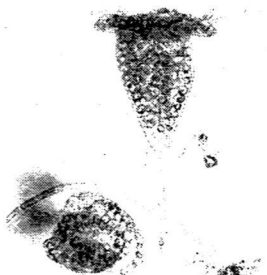


Rotifers



Nauplius larva

## Protozoans



*Vorticella* sp.

## Reference

- Yatigammana, S. and Perera, M.B.U., A Guide to Common Planktons of Sri Lanka, 2009, Department of Zoology, University of Peradeniya.
- Nollet, L.M.L., Hand book of water analysis, 2000, C.H.I.P.S. publishers, Texas.

# High Performance Liquid Chromatography

Lalith Jayasinghe

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Chromatography is the general term for a set of techniques used for the separation of compounds, to isolate pure compounds from a mixture of compounds. This technique involves two phases, the mobile phase and the stationary phase. High Performance Liquid Chromatography (HPLC) is one such technique which can be applied for the separation and isolation of all classes of natural products. The main difference between HPLC and other column chromatographic methods is the smaller particle size (10-3 μm) of the packing material in HPLC columns. Hence high pressure is required to pass the compound mixture through the column.

HPLC analysis can be of two types - analytical chromatography and preparative chromatography. Analytical chromatography involves small amounts of the target molecules necessary for either comparison with an authentic sample or to measure the relative abundance of the compounds in a mixture. Preparative chromatography involves the larger scale isolation of compounds for further use.

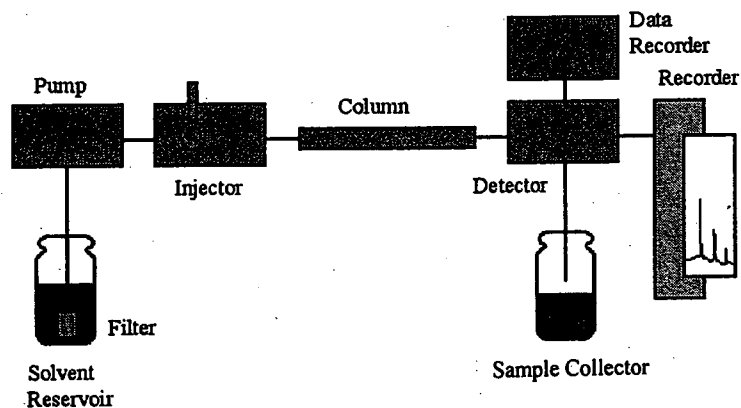


Fig. Basic configuration of an HPLC system

The choice of the packing material is decided by the physical and chemical properties of the target molecules. A wide range of packing materials are available. eg., silica, C<sub>18</sub> (octadecyl - ODS), C<sub>8</sub> octyl, C<sub>6</sub> hexyl, C<sub>2</sub> ethyl, C<sub>1</sub> methyl, Ph phenyl, CH cyclohexyl, CN cyanopropyl, 2OH diol, aminopropyl, benzenesulphonic acid, quaternary ammonium etc.

Chromatographic eluents comprise mixtures of only organic solvents, or organic solvents and water, sometimes with buffers, acids, bases etc. Choice is dependent on the compatibility with the stationary phase. For gradient elution second and third

pumps may be used. Solvents may be passed through the column under constant flow or constant pressure.

The sample to be separated is introduced at the top of the column through the injector by an HPLC syringe, which is specially designed with a flat tip. First the sample is filled into a sample loop that is temporarily switched away from the flow of eluent. The volume of the sample loop may vary from 10 - 500  $\mu$ l.

The detector is the component that gives a response to the recorder when the sample passes through the detector. UV detectors are popular among users due to their high sensitivity, and are used to isolate compounds which absorb in the range of 190 - 400 nm. The majority of organic compounds can be analyzed by UV detectors and most HPLC analyses are performed using UV detectors.

The UV diode array detectors allow the collection of UV absorbance data across many wave lengths simultaneously. Hence the user can save a considerable amount of time in analyzing compounds. The second widely used detector is the RI detector (Refractive Index), which is less sensitive than a UV detector. Analysis of compounds which occur at low levels by RI, may create problems. A further disadvantage of this method is that it is limited only to the use of isocratic solvent systems. In selecting eluents for HPLC analysis, it is important to have an idea about the UV cut off values and the refractive index of solvents.

An HPLC is an expensive instrument. Hence extra attention should be paid to maintain the instrument especially by using degassed and filtered solvents, and cleaning columns properly after each analysis. However many mixtures of compounds separated by HPLC contain significant amounts of compounds that bind strongly with the column material. This will reduce the performance efficiency of the column after sometime. Hence regular cleaning of columns is important. To minimize the damage to the HPLC column, a small guard column, with the same stationary phase packing, may be fixed to the main column.

## PROCUREMENT PROCESS & DOCUMENTATION

*P. Rajanathan*

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- **PROCUREMENT ?**
  - Obtain, Acquire, Get, Pick up, Fnd, Secure..... (Source: Oxford Dictionary)
- Procurement includes the acquisition of both goods and services, thus embracing not only purchasing-buying of goods – but also the hiring of contractors or consultants to carry out services
- It's a process by which the Goods, Works, Other services and Consultant Services are acquired.

- **GOODS:** Commodities, raw materials, equipments, products, and other physical objects of every description, in solid, liquid or gaseous form.
- **WORKS:** All construction work associated with the construction, reconstruction, demolition, repair or renovation.
- **OTHER SERVICES:** Other than Consultant Services.
- **CONSULTANT SERVICES:** Means the services provided by an individual or a firm using their expert knowledge.

- **Private sector:** Flexible
- **Public Sector:** Need to follow more rules and regulations
  - Public Finance (Guidelines, Manual, Circulars...)
  - Government Tender Procedure (1997, Re. 2000)
  - National Procurement Agency (NPA) – 2005
  - Government Procurement Guidelines 2006 (Goods & Works)
  - TRANSPARENCY** – Bidders Information Confidentiality

**S1+P4+M2+T5+B2+C1 = PROCUREMENT**

- S1 = Scope (Goods / Works / Services/Consultancies)
- P4 = 1- Policy (Cost, Time –Economy and Efficiency, Transparency, Equal Opportunities) +
  - 2- Packaging (Similar Items, Market survey)
  - 3 - Planning (Procurement Method, Estimated Cost, Required Date, Order completion, Schedule/Revision)
  - 4- Procedure (GOSL- GTP / NPG)
- M2 = 1- Methods of Procurement – Goods (ICB, NCB, LIB, LNB, NS, IS & DC)
  - 2 – Methods of Selection – Consultancy (QCBS, QBS, FBS & SBCQ, LC, SSS-IS)
- T5 = 1 - Types of Contracts (Fixed Price, Measure, Re-imburse, Turnkey, Design & Build, Lump sum, Time based)
  - 2 - Thresholds (NS / NCB)
  - 3 - Technical Specifications (Direct / Dimensional / Material / Performance / Standards – Brand names not allowed , Drawings, Test Certificate, Warranty)
  - 4 - Time Frame
  - 5 - Terms of Reference (Background, Scope, Objectives, Tasks, Output, Qualification, Duration, Location, Facilities, Payment, Reporting obligation)

**S1+P4+M2+T5+B2+C1 = PROCUREMENT**

- B2 = 1- Bidding Documents (Invitation for Bid, Instruction to Bidders, Bid data Sheet, General Conditions, Special conditions, Schedule of requirements, Technical Specifications, Bid Price Schedule)
- 2 - Bid Evaluation – "Lowest Evaluated Responsive and Eligible Bid"
  - Bid Opening, Red out Prices, Bid Opening Minutes, Late Bids, Eligibility, Bid Security, Responsiveness, Errors, Discounts, Currency, Compliance with Specifications – Terms and Conditions, Post Qualifications, Bid Price, Delivery Period/ Point, Payment Schedule, Cost of Accessories and Spare parts, After Sales Service and Spares, Efficiency, Recommendations
- C1 = Contract Management – Preparation of Contract, Documents, Signing Advance Payments, Part Payment, Establishing Letter of Credit, Delays in performance, Inspection of Certificate, Stock Control / Inventonization, Payment

- SHOPPING
- Shopping is an appropriate procurement method for procuring frequently used Items
- Readily available off the shelf Goods or small value items
- Small value works & Services
- Telephone / Verbal quotations are not acceptable
- Registration of Suppliers
- Inviting Bids (IFB) – Notice Time (14 days) / Bid Validity (90 days)
- Bid Opening
- Evaluation – TEC Recommendation

- Procurement Committee Approval
- Letter of Acceptance / Purchase Order
- Follow Up – Delivery
- Installation / Test Run
- Goods Received Note / Inventorization
- Stores Management / Stock Control
- Fixed Asset Coding
- Payment

- INTERNATIONAL SHOPPING
- Inviting Bids (IFB) –IN COTERMS / CCCN ( 21 Sections – Sec. 6-Chp.-28 - IOC ,29 – OC, Sec 13- Chp. 70 – G & GW, Sec 18-Chp. 90-LE)
- Bid Opening
- Evaluation / TEC Recommendation
- Procurement Committee Approval
- Bank payment – Draft / Telegraphic Transfer / Letter of Credit
- Shipping Details – Bill of Lading, Air Waybill
- Receipt of Shipping Documents
- Shipping Endorsement

- Customs Clearing – Taxes etc.,(CIF 2.5%, Surcharge 10%, Vat 15%, PAL 3%, SRL- Social responsible Levy 1%, NBT 3% = 34.5%)
- Delivery
- Installation / Test Run
- Goods Received Note / Inventorization
- Stores Management / Stock Control
- Fixed Asset Coding

- NCB – NATIONAL COMPETITIVE BIDDING
- Paper Advertisement – Procurement Notice
- Tender Documents
- Conditions / Requirements / Specifications / Price Schedule / Bid Security 2% estimated Cost / Bid Validity 91 days
- Bid Opening
- Evaluation
- TEC Recommendation
- Procurement Committee Approval
- Letter of Acceptance / Award of Contract
- Performance Security – 10% of contract value
- Warranty period – ( 8 %/ 5 % / Balance )

- Delivery - Liquidated damages
- Inspection
- Installation / Test run
- Goods Received Note / Inventorization
- Stores Management / Stock Control
- Fixed Asset Coding
- Payment

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- DELIVERY OF GOODS
- Suppliers Invoice
- Delivery Note
- Manufacturers or Suppliers Warranty Certificate
- Manufacturers Factory Inspection Certificate
- Certificate of Origin

- Procurement Plan
- NS IFB
- NS IFB 2
- International Shopping IFB
- INCOTERMS - BTN
- Bidders Response to Specification 1
- Bidders Response to Specification 2
- Lab Equipment Schedule
- BID OPENING MINUTES
- BID EVALUATION
- Letter of Acceptance
- Inventory Certificate

- Procurement Policy
- Procurement Plan
- Requirement List / Specifications - TEC
- IFB – NS / IS / NCB
- Bid Opening
- Bid Evaluation
- TEC Approval
- RPC Approval
- Delivery
- Stores Management
- Document filing

- Department of Chemistry – UOP
  - USAID Project
  - WHO Project
  - IPICS, Uppsala University & IFS, Sweden
  - SAREC SIDA Project
  - CIBA GEIGY Project
  - National Science Foundation
  - National Science Council
  - National Research Council
- IRQUE – World Bank Credit 3781CE
  - Policy Planning Development Unit – PPDU
  - Local Technical Secretariat – LTS
- Professor (Mrs.) N. S. Kumar