# CHAPTER II

# LITERATURE REVIEWS

**Sample preparation** [7, 13]

Elemental analysis of the majority of organic and inorganic matrices requires the partial or total dissolution of the sample prior to instrumental analysis. Only a few direct methods allow the introduction of the sample without any preparation, as presented in Table 1. In these cases the lack of reliable calibration is the major problem. On the other hand, sample preparation allows the separation and/or pre-concentration of analytes and makes possible the use of several determination methods (Table 1) [13].

**Table 1 Relationship between sample preparation and determination methods** [13]

| | |
|---|---|
| Methods that allow direct sample analysis | X-ray Fluorescence<br>Neutron Activation<br>Thermogravimetry |
| Methods that need sample preparation (separation and pre-concentration) | Gravimetry<br>Titrimetry<br>Spectrometry<br>Electroanalysis<br>Chromatography |

Nowadays, the goals to be accomplished are the best results, in the shortest time, with minimal contamination, low reagent consumption and generation of minimal residue or waste. In order to achieve these objectives of the analysis, some aspects of sample preparation should be taken into account, focusing on the chosen procedure. Thus, simplification in sample manipulation, use of high purity water and reagents in suitable amounts, correct cleaning of recipients and blank preparation in

parallel to the samples are desirable. In addition, the validation of the methodology is important, usually with certified reference materials [14, 15, 16, 17].

Sample preparation can be performed by simple dilution, or with heating for pre-concentration or partial or total dissolution. However to achieve most sample preparations, it is necessary to add reagents to the sample and to apply enough energy to break some bonds and the crystalline structure of solids. If necessary, it is possible to use complementary reagents to obtain the analytes in solution. Two basic procedures are normally used for sample preparation: dry and wet decomposition [14, 15, 18, 19].

**1. Dry decomposition**

Silicates, refractory materials, some mineral oxides and iron alloys were normally decomposed by fluxes (fusion processes). The sample was mixed with a flux and then fused to form products that could be dissolved in water or diluted acid. The high temperature (300 to 1000 °C) by flame, conductive or microwave-assisted heating was required for this procedure. Some examples for fusion sample preparation were presented in Table 2. The drawbacks of this method were the contamination in the fluxes, the high amounts of electrolytes in the resulting solution, especially in the case of some analytical spectroscopic techniques, risk of contamination and losses by volatilization [14, 15, 16, 19, 20].

**Table 2 Fusion sample preparation** [21]

| | |
|---|---|
| Temperature | 300 - 1000 °C |
| Fluxes | Lithium metaborate<br>Sodium carbonate<br>Potassium nitrate<br>Alkaline hydroxides |
| Crucible | Platinum, nickel, silver, iron,<br>graphite, porcelain |
| Disadvantages | High content of dissolved solids |

Dry ashing [14, 15, 16] is used for the elimination or minimization of organic materials, before mineral element determination, and consists of the ignition of the organic compound in a stream of oxygen or in air. To avoid volatile element losses such as As, Cd, Hg and Pb, some additives can be added. Some examples of additives for dry ashing sample preparation are presented in Table 3 [21].

**Table 3 Additives for dry ashing sample preparation** [21]

| To avoid losses of: | Additives |
|---|---|
| Volatile elements (As, Hg, Pb) | Magnesium nitrate |
| Volatile chlorides (Zn, Sn etc.) and volatile elements | Sulfuric acid |
| Temperature | 500-800 °C |

**2. Wet decomposition**

For wet decomposition, oxidizing agents were normally employed to decompose the organic samples before to metal content determination or to extract metals from inorganic matrices, allowing their determination. Typically, concentrated acid plus heating were used. The strength of the acid, oxidizing and complexing power, boiling point, the solubility of the resulting salts, safety in manipulation and purity were important aspects to consider [14, 22, 23]. For open vessel decomposition, the boiling point of the acid controls the maximum temperature for decomposition in order to avoid high acid consumption and plausible losses of the volatile elements [14, 15, 16]. For organic samples, metallic alloys, common minerals, soils, rocks, clays and silicates, nitric acid, hydrochloric acid, sulfuric acid, phosphoric acid, hydrofluoric acid and hydrogen peroxide as well as mixtures of such reagents were used.

Hydrofluoric (HF) or perchloric acids were carefully and used special care should be taken. PTFE or other plastic materials were used as containers when HF was used. It should be emphasized that contact of HF with skin results in serious damage. Perchloric acid was normally used in organic matter but it should never be more

concentrated than 72% owing to the risk of explosion. More details can be found in references 21. Wet digestion could be carried out using different forms of energy: thermal, ultrasonic and radiant (infrared, ultraviolet and microwave).

**2.1. Thermal energy**

The conventional approach to wet digestion, which has proven its worth over many years, entails a system equipped with heated conventional source (Bunsen, heating plate, sand bath, digestion block, oven or muffle) operating either at a fixed temperature or in response to a temperature program. Acid digestions are often accomplished in any vessel, usually in glass or PTFE (beaker, conical flask) with or without a refluxing condenser. However, when a sample is decomposed by open wet digestion, refluxing is compulsory. The necessary apparatus has been described by Welna and Twyman [16, 24]. Open block digestion systems have been popular in sample analysis over the past decades, but have consistently suffered from the major drawback of their sensitivity against corrosion and subsequent risk of contamination. Therefore, block digestion systems (hotplate techniques) have not been considered state-of-the-art technology in trace and ultratrace sample preparation. Graphite block digestion systems are becoming more frequently considered. These systems overcome the deficiencies of the traditional systems, made from stainless steel or aluminum, because the block is manufactured from graphite and typically coated with a fluoropolymer to prevent the possibility of metallic contamination from the surface of the system during the handling of the samples. Graphite block systems present an alternative to the current mainstream technology of open and closed vessel (classical or microwave-assisted) digestion systems, as they allow large numbers of samples to be digested simultaneously, thus overcoming one of the major weaknesses of closed vessel systems. Commonly employed digestion agents include nitric acid, sulfuric acid, hydrofluoric acid, perchloric acid and hydrogen peroxide, as well as various combinations of these. Most applications of wet digestion involve aqueous or organic matrices, such as surface water, waste water, biological and clinical samples, food samples, as well as soil, sediment and sewage sludge, coal, high purity materials and various technical materials. More recently, open systems have progressed: the usual digestion ramps consist of several vessels equipped with reflux condensers to limit possible volatilization losses of some analytes and to avoid the evaporation of the

reactive mixture. Such assembling is entirely satisfactory for ensuring concurrent digestions of large series of samples. The main problems to consider are: time consumption (hours), the use of large amounts of reagents, contamination from the environment, pre-concentration of reagent impurities, the use of strong oxidizing agents, such as hydrogen peroxide, and the need of constant supervision. The analyst also should be well prepared. For some refractory materials, such methods are not adequate. Even so, for many cases, reliable results can be achieved. Examples are presented in Table 4 [21].

**Table 4 Examples of wet dissolution by using thermal energy for several ores** [21]

| Samples | Analytes | Reagents |
|---|---|---|
| Silicates | Traces | $HF + H_2SO_4$ |
| Chromium ore | Traces | $H_2SO_4 + HCl + NaCl$[a] |
| Magnetite | Ge | $H_3PO_4 + KMnO_4$ |
| Pyrite | Traces | $HCl + HNO_3$ |
| Magnesium ores | Traces | HCl |
| | | (digestion of the residue) |
| Monasite | Ln[b] | $H_2SO_4 + H_2O_2$ |
| Fluorite | Traces | $H_2SO_4 + HCl$ |
| | | (digestion of the residue) |
| Mercury ores | Traces | $HCl + HNO_3 + H_2SO_4$[c] |
| Bauxite | Traces | $HCl + HNO_3 + H_2SO_4$ |
| Sulfide ores | Cu | $H_3PO_4 + HCl$ |

[a] NaCl is used to form and remove volatile $CrOCl_2$;

[b] Ln : lanthanide elements;

[c] Evaporation of the $HgCl_2$.

Modern commercially available such as "thermoreactor" is designed to digest organic and mineral samples for subsequent analysis.

Thermoreactor (Figure 1) [8, 25, 26] is mainly composed of an induced heating coil made of ferromagnetic materials and control system. In principle, current generates a powerful magnetic field via the coil. Large numbers of vortexes are produced in the way that the magnetic lines in the field contact the bottom containing iron or stainless steel substances. The tremendous energy generated by the vortexes can heat the sample in digestion tube directly by converting efficiency heat energy penetrating through the digestion block. Thermoreactor is an apparatus which is able to perform a variety of analyses providing highly accurate results. It has been studied for COD analysis and for sample preparation, by wet digestion, for the determination of metallic and non metallic elements on different organic and inorganic samples. The thermoreactor allows setting and controlling of time and temperature with high precision and reproducibility. So, this apparatus was chosen in this study for generating of thermal energy in the digestion procedure.

**Figure 1 Thermoreactor** [8]

### 2.2. Ultrasonic energy

Ultrasound-assisted metal extraction was proposed as a simple and inexpensive alternative for sample preparation for biological and inorganic matrices. The extraction effect is considered as being caused by acoustic cavitation, that is, bubble formation and subsequent disruptive action. The collapse of bubbles, created by sonication of solutions, results in the generation of extremely high local temperature and pressure gradients, which help sample preparation [21, 27].

A diluted acid medium is normally used, thus decreasing blank values and reducing both reagent and time consumption, as compared to traditional wet digestion using conductive or microwave-assisted heating. Also a smaller sample amount is used [21, 27]. This method, using a bath system, allows the preparation of several samples directly in the sample container, preventing sample losses and minimizing contamination, and is mainly used for extraction of the analytes. Ultrasound probes are generally employed for decomposition of organic compounds in environmental samples. Examples are presented in the literature for sample pretreatment [28-29] and also for synthesis [30].

### 2.3. Infrared radiation

Infrared-assisted element extraction has been employed for organic and biological samples [31, 32]. Infrared radiation ($1.2\times10^{-14}$ to $6.0\times10^{-12}$ Hz) causes an increase in molecular vibration and variation in molecular rotation, generating heat and also its propagation. The applications are, for example, the heating of liquid and solid samples for determination of volatile analytes [21], and for the preparation of sub-boiling acids [33].

### 2.4. Ultraviolet radiation

Ultraviolet (UV) radiation decomposition devices are used for complete removal of organic materials, normally when polarography or voltametry is used for subsequent elemental determination [21]. UV digestion is utilized mainly in conjunction with uncontaminated or slightly contaminated natural water matrices (aqueous solutions), such as sea, surface, fresh, river, lake, ground, estuarine and coastal water. Liquids or slurries of solids are decomposed by UV radiation (light) in the presence of small amounts of hydrogen peroxide, acids (mainly $HNO_3$) or peroxodisulfate (i.e., beverages, special industrial waste water, water of sewage treatment plants, soil extracts) [34]. Dissolved organic matter (DOM) and complexes of the analyte elements are decomposed to yield free metal ions. The corresponding digestion vessel should be placed in the closest possible proximity to the UV lamp (low- or high-pressure) to ensure a high photon flux. In photolysis, the digestion mechanism can be characterized by the formation of $OH^{\bullet}$ radicals from both water and hydrogen peroxide that is initialized by the aid of the UV radiation [34]. These reactive radicals are able to oxidize, to carbon dioxide and water, the organic matter

present in simple matrices containing up to about 100 mg $L^{-1}$ of carbon. Complete elimination of the matrix is, of course, possible only with simple matrices or by combining photolysis with other digestion techniques [35]. The method does not oxidize all organic components possibly present in water; chlorinated phenols, nitrophenols, hexachlorobenzene and similar compounds are only partly oxidized. Effective cooling of the sample is essential, since losses might otherwise be incurred with highly volatile elements. Hydrogen peroxide addition may need to be repeated several times to produce a clear sample solution. Modern UV-digestion systems are commercially available [34].

**2.5. Microwave radiation**

Sample preparation by microwave-assisted digestion was used for a wide range of applications, including decomposition of inorganic and organic materials [18, 19, 23, 36, 37, 38]. Both ionic migration and dipole rotation, resulting in rapid heating of the mixture with subsequent decomposition were caused by interacting of microwave radiation (2450 MHz) with sample and reagents. The short time were required to perform decomposition of the sample, direct heating of samples and reagents (the vessels are only indirectly heated by the hot solution), minimal contamination and no loss of volatile elements were the main advantages of this method. The blank signal was decreased by using small amounts of reagents.

In the recent year, the advent of sample digestion techniques was performed on the basis of the usage of UV radiation coupled with oxidizing agent. There has been an effective improvement on the sample pretreatment. However, in this study oxidizing acid is concerned because NR latex will coagulate when acid is added causing difficulty in the digestion procedure. Thus, $K_2S_2O_8$ and $(NH_4)_2S_2O_8$ were attended to the NR latex digestion. The widespread applications of UV digestion coupled with peroxodisulphate were reported in the decomposition of several samples [9, 10, 12, 39]. Benson and coworkers [9] employed a UV photoreactor and a thermal digestion unit connected in an on-line flow-injection (FI) system for the determination of total phosphorus in waters and wastewaters. The mixture of $(NH_4)_2S_2O_8$ and perchloric acid was used as the oxidizing reagent for decomposition of matrices in the water samples. The proposed FI UV/thermal digestion procedure for total phosphorus has been shown to be rapid, effective and safe. $K_2S_2O_8$ in acidic medium was used as

the oxidizing agent in an on-line UV photo-oxidation method for the determination of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) after convertion to $CO_2$ in freshwaters. The results showed good agreement with a high temperature catalytic oxidation (HTCO) reference method [10]. Paraskevas and coworkers [12] used $(NH_4)_2S_2O_8$ as the oxidizing reagent coupled with UV-assisted digestion in an on-line spectrophotometric determination of fosinopril (FSP), a phosphorus-containing compound, employing the molybdenum blue approach. The application of the developed flow injection method to two FSP containing pharmaceutical formulations gave very precise and accurate results. Besides, Zhang and coworkers were applied an on-line UV photo-oxidation digestion using $K_2S_2O_8$ as oxidizing agent for the measurement of arsenic in serum of uraemic patients based on liquid chromatography with hydride generation atomic absorption spectrometry (HGAAS). The $K_2S_2O_8$ showed high efficiency for decomposition of matrices in human serum. Obviously, the use of peroxodisulphate ions in the digestion of NR latex becomes possible. In addition, peroxodisulphate ion could be decomposed under photolysis or heat conditions to generate highly reactive species such as sulfate radicals ($SO4^{\bullet -}$) and hydroxylradicals ($OH^{\bullet}$) initiating a series of radical chain reactions (Figure 2) [12]. To the best of our knowledge, there was no report on the use of UV radiation/thermal energy coupled with peroxodisulphate ion for the digestion of NR latex. Therefore, in this work UV digestion and thermal digestion coupled with peroxodisulphate ion will be studied for sample pretreatment before determination of total phosphorus in NR latex.

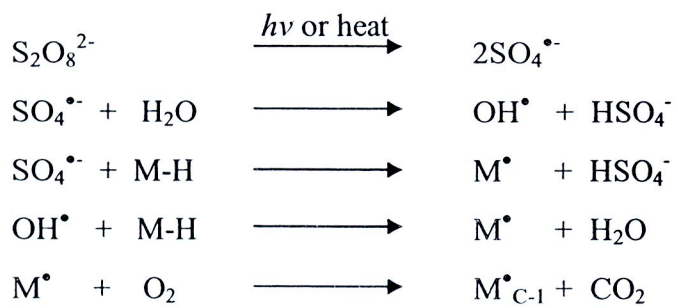


**Figure 2 Decomposition reaction of peroxodisulphate ion under photolysis or heat conditions** [12]

**Natural rubber latex**

Natural rubber (NR) latex is obtained from the botanical source *Hevea brasiliensis* and is the name commonly used to denote a stable colloidal dispersion of a polymeric substance of *cis*-1,4 polyisoprene of high molecular mass in an aqueous medium [1]. The exact structure of NR is still unknown. Early X-ray diffraction studies showed that the double bonds of the isoprene repeat units are in *cis* configuration [40]. By the use of $^{1}H$ and $^{13}C$-NMR spectroscopies, Tanaka [41] later showed that the second and third units of *Hevea* rubber are *trans*, followed by repetitive *cis* enchainment (Figure 3) [41]. The terminal groups are believed to be - $CH_2OH$ or a fatty acid ester. NR latex is an exudation of the plant's cytosol, it contains many of the substances found naturally in the tree: carbohydrates, proteins, fats, and inorganic and organic salts [42, 43].

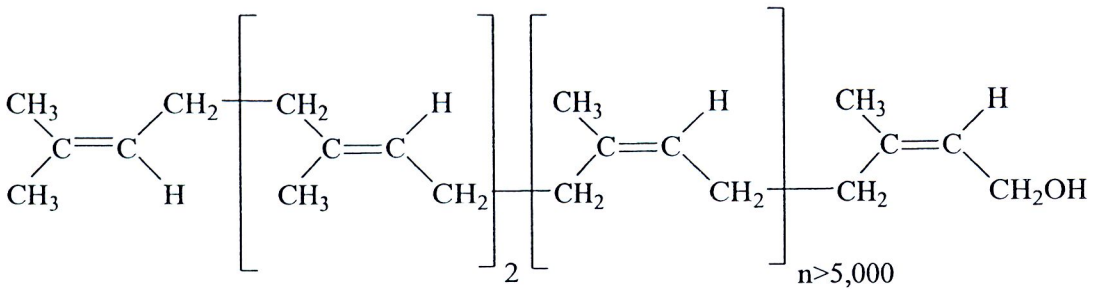


**Figure 3 The microstructure of natural *Hevea* rubber** [41]

**Phosphorus in NR latex** [4]

Latex, which is a type of biotic liquids, can be deteriorated if it is not preserved by ammonia or sodium sulfite which is called anticoagulant. Anticoagulants prevent latex from pre-coagulation. The kind of anticoagulant used is depended upon the production process. Sodium sulfite is preferred if crepe or sheet rubbers are to be made, but ammonia is more suitable for latex concentrate. Ammonia is able to de-activate some multivalent metal ions through sequestration by the formation of amine ions. Other multivalent metal ions are effectively de-activated through removal from the latex aqueous phase by precipitation as hydroxides. More important for the colloid stability of ammonia-preserved natural rubber latex is the interaction of magnesium ions with ammonia and any orthophosphate anions which may be present in the

aqueous phase, to precipitate the very sparingly-soluble magnesium ammonium phosphate according to the following reaction [4]:

$$Mg^{2+} + NH_3 + HPO_4^{2-} \longrightarrow MgNH_4PO_4 \downarrow \quad (1)$$

For the precipitation of magnesium ammonium phosphate to occur, it is necessary that sufficient orthophosphate anions be present in the latex aqueous phase. These anions are principally derived by alkaline hydrolysis of phospholipids present in the fresh latex. Some NR latex do not contain sufficient phosphate to enable the magnesium to be completely precipitated in this way. The magnesium/phosphate ratio is to some extent a clonal characteristic, although there can be large variations within a given clone. Some clones, give a latex for which this ratio is high. The ammonia-preserved latex concentrate tends to be of low and variable colloid stability because of the relatively high concentration of residual magnesium ions. The mechanical stability of the concentrate is low, its viscosity at a given solids content is high, and its gelling pH is high. These abnormalities, which are accentuated during the wintering period, are unfortunate because the clone is particularly high-yielding.

Adverse magnesium/phosphate ratios are usually the consequence of an excess of magnesium, rather than of a deficiency of phosphate. Indeed, it is significant that the abnormal lattices are not deficient in the phospholipids which are believed to be the principal source of orthophosphate anions. It has shown that small additions of water-soluble phosphates, such as diammonium hydrogen phosphate or sodium pyrophosphate, improve the mechanical stability of latex, up to the point where the added phosphate is roughly equivalent to the residual magnesium. Further additions of water-soluble phosphate cause the mechanical stability to decrease again, presumably because the ionic strength of the aqueous phase is increased. The addition of water-soluble phosphates to a normal NR latex concentrate brings about an immediate reduction in mechanical stability. Addition of water-soluble phosphates to latex also reduces the gelling pH to a normal value.

Phosphorus occurs in NR latex as free orthophosphate, sugar phosphates and phospholipids [4]. It is also added to the latex as diammonium phosphate to precipitate magnesium phosphate which have a high magnesium content, prior to centrifugation,

and thus improve the stability of that latex. Unfortunately, if too much phosphate is added the stability will decrease again; thus in any situation where the stability of a NR latex is suspect, the phosphorus content should be determined. However, there is a study on the effect of phosphate on the stability of concentrated NR latex such as mechanical stability time, volatile fatty acid number and chemical stability time and properties dipped products such as aged and unaged tensile properties [2]. The latex with a phosphate ion concentration of 30 ppm was found to produce the best-quality latex and dipped products.

In order to be certain of analyzing for the total phosphorus content, a dried film should first be prepared from the latex, care being taken to adhere to the sampling procedures. The non-instrumental analysis of a dry rubber sample for phosphorus content can be divided into two parts; first, the removal of the polymer and the production of a solution of phosphate ions by sample digestions, and second the development of a color which can be measured spectrophotometrically and the intensity of which is proportional to the concentration of phosphorus [4].

**Phosphorus assay techniques** [6]

Spectrophotometric methods often require the initial conversion of phosphorus to soluble orthophosphates which can then be determined colorimetrically. Orthophosphates can be analyzed quantitatively by three methods: the vanado-molybdophosphoric acid method, the stannous chloride method and ascorbic acid method.

**1. The vanadomolybdophosphoric acid method** [6]

In a diluted orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to phosphate concentration. The wavelength at which color intensity is measured depends on the sensitivity desired, because the sensitivity varies tenfold with wavelengths 400 to 490 nm. Ferric iron causes interference at low wavelengths, particularly at 400 nm. A wavelength of 470 nm usually is used. The chemical equations are as follows:

$$PO_4^{3-} + 12(NH_4)_2MoO_4 + 24H^+ \longrightarrow (NH_4)_3PO_4 \cdot 12MoO_3 + 21NH_4^+ + 12H_2O \quad (2)$$

**2. The stannous chloride method** [30]

Soluble orthophosphate ($PO_4^{3-}$, and its associated forms: $HPO_4^{2-}$, $H_2PO_4^-$ and $H_3PO_4$) can be treated to produce a blue colored compound. To measure other forms of phosphorus, organic phosphorus, or condensed phosphates, first requires conversion of these forms to orthophosphate. Condensed phosphate can be converted to orthophosphate by boiling the sample after the addition of acid. Organic phosphorus can be converted to orthophosphate by adding an oxidizing agents and acid then digesting the sample. If a sample is digested under these conditions, the condensed phosphates will also be converted to orthophosphate. If particulate phosphorus is to be distinguished from soluble phosphorus, the sample should be filtered and separate analyses performed on an unfiltered and filtered aliquot. When ammonium molybdate and stannous chloride are added to a sample containing orthophosphate, a blue colored compound (molybdenum blue) develops. This compound absorbs light of wavelength 690 nm most strongly. The colored compound is not stable, and the light absorbance must be measured between 10 and 12 minutes after reagent addition. The chemical equations are as follows [30]:

$$PO_4^{3-} + 12(NH_4)_2MoO_4 + 24H^+ \longrightarrow (NH_4)_3PO_4 \cdot 12MoO_3 + 21NH_4^+ + 12H_2O \quad (3)$$

$$(NH_4)_3PO_4 \cdot 12MoO_3 + Sn^{2+} \longrightarrow \text{Molybdenum Blue} + Sn^{4+} \quad (4)$$

**3. The ascorbic acid method** [6, 44]

Three techniques for colorimetric analysis of phosphorus are available [6]. The technique most commonly used is the ascorbic acid method, which can determine concentrations of orthophosphate in most waters and wastewater in the range from 2-200 μgP $L^{-1}$. Ammonium molybdate and antimony potassium tartrate react in an acid medium with diluted solutions of orthophosphate-phosphorus to form an intensely colored antimony-phospho-molybdate complex compound. This complex compound is reduced to an intensely blue-colored complex compound which can be measured in a spectrophotometer at 880 nm wavelength by ascorbic acid. The color is proportional to the phosphorus concentration. The complex compound is not stable and thus analysis

must be performed within 30 minutes of adding the ammonium molybdate and antimony potassium tartrate [44]. The chemical equations are as follows [45]:

$$PO_4 + 12(NH_4)_2MoO_4 + 24H^+ \longrightarrow (NH_4)_3PO_4{\cdot}12MoO_3 + 21NH_4 + 12H_2O \quad (5)$$

$$(NH_4)_3PO_4{\cdot}12MoO_3 \longrightarrow \text{molybdenum blue} \quad (6)$$

For interfering ions of the three methods, arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphorus. Concentrations as low as 0.1 mgAs $L^{-1}$ interfere with the phosphorus determination. Hexavalent chromium and $NO_2^-$ interfere to give results about 3% low at concentrations of 1.0 mg $L^{-1}$ and 10 to 15% low at 10.0 mg $L^{-1}$. Sulfide ($Na_2S$) and silicate do not interfere at concentrations of 1.0 and 10.0 mg $L^{-1}$. In order to determine phosphorus, it is necessary to convert all forms of phosphorus to orthophosphate. This can be done by the peroxodisulphate digestion method in which the sample is heated with sulphuric acid and either ammonium or potassium peroxodisulphate [6].

Selection of the method for phosphorus determination depends largely on the concentration range of phosphorus. The vanadomolybdophosphoric acid method is most useful for routine analysis in the range of 1 to 20 mgP $L^{-1}$. The stannous chloride method or the ascorbic acid method is more suited for the range of 0.01 to 6 mgP $L^{-1}$. An extraction step is recommended for the lower levels of this range and when interferences must be overcome. Automated versions of the ascorbic acid method also are presented [6]. Careful attention to procedure may allow application of these methods to very low levels of phosphorus.

In this work, the ascorbic acid method was chosen for color developing of phosphorus in NR latex because the sensitivity of this method is higher than that of the vanadomolybdophosphoric acid method and more environmental friendly than the stannous chloride method.

**Ultraviolet and Visible (UV-Vis) spectroscopy** [23, 46, 47]

UV-Vis spectrometry provides a technique that may be used to detect one or more components in a solution and measure the concentration of these species. The primary advantage of this technique is that even traces of substances can be

determined in a simple way which is not possible with classical analytical methods like gravimetric and volumetric procedures. In addition, UV spectrometry is also used for obtaining structural information of substances; particularly the organic compounds and it may as well help in establishing the identity of a molecule. This technique can be applied in qualitative and quantitative in chemistry.

**Instrumentation for UV-Vis spectrophotometry**

Today a wide range of instruments are available for making molecular absorption measurements in the UV-Vis range. These vary from simple and inexpensive machines for routine work to highly sophisticated devices that can be used for specialized work and of course the routine jobs also. However, the basic components of these instruments remain the same. The five essential components of UV-Vis instruments are as follows:

1. A stable radiation source
2. Wavelength selector
3. Sample holder
4. Radiation detector
5. Signal processing and output device

The general layout of the essential components in a simple absorption instrument is given in Figure 4.

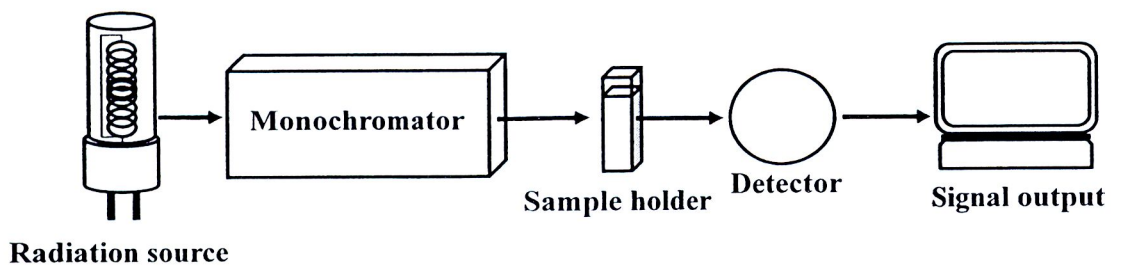


**Figure 4 General layout of the essential components in a simple absorption instrument** [47]

**1. Radiation Sources**

A spectrophotometric radiation source must provide a stable high energy output over a broad range of wavelengths. There is no inexpensive source available

that may provide stable output over the entire UV-visible range (190 nm to 780 nm). The radiation sources commonly used for the UV and visible range are described below and shown in Figure 5.

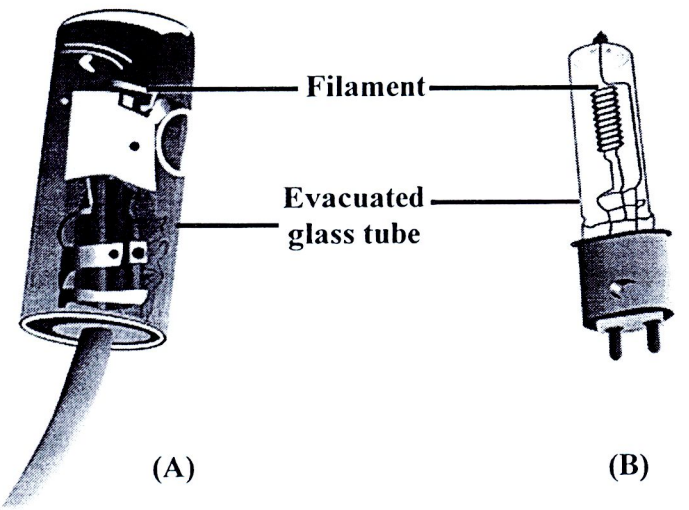


**Figure 5 Radiation sources: deuterium lamp for UV range (A) and tungsten lamp for visible range (B)** [47]

**Sources for UV region** [47]

For measurements in the UV region, electric discharge sources like hydrogen or a deuterium lamp are used. In these, the excitation of the gaseous molecules is brought about by the passage of electrons through the gas at low pressures. A hydrogen lamp is commonly used in the spectrophotometers and gives light in the wavelength region of 160-375 nm. The radiant power of the hydrogen lamp is low and these are replaced by deuterium lamps but it increases the cost of the instrument.

**Sources for visible region** [47]

In the beginning of development of instruments for colorimetric work, sunlight was used as source for measurements in the visible range. The modern instruments use a tungsten filament lamp as the radiation source. This consists of a thin, coiled tungsten wire that is sealed in an evacuated glass bulb. This gives radiations in the range of 350-2200 nm. As the output depends on the voltage, the tungsten lamp is energized by a 6 or 12 volt storage battery or by the output of a constant voltage transformer.

Nowadays, some instruments use tungsten-halogen lamps that contain a small amount of iodine in the quartz bulb housing the tungsten filament. The presence of iodine extends the output wavelength range of the lamp from 240-2500 nm.

**2. Wavelength selectors** [47]

In spectrophotometric measurements, a narrow band of wavelengths of light is used. This enhances the selectivity and sensitivity of the instrument. Less expensive instruments use a filter to isolate the radiant energy and provide a broad band of the wavelengths. The continuously vary the wavelength over a defined range was needed in many applications. This can be achieved by using monochromators. Most modern instruments use monochromators that employ a prism or diffraction grating as the dispersing medium. The selection and use of fillers and monochromators is described as the following.

**2.1 Absorption filters**

In low cost instruments catering to measurements in the visible range, colored glass filters are used to cut off undesirable wavelengths. A typical filter is a colored piece of glass, which absorbs light of certain wavelength and allows that of the other to pass through. The white light is made up of seven different colors; the acronym is VIBGYOR for violet, indigo, blue, green, yellow, orange and red. These seven colors add up to give white light back. When white light falls on an object, a part of is absorbed and rest is transmitted. These transmitted components add up to give the observed color of the object. The absorbed component and the observed color can again add up to give white light. These are therefore called as complementary to each other. An object of a particular color looks of that color because this color is transmitted and its complementary color is absorbed.

The selection of a suitable filter for a measurement of absorbance of a solution is based on the complementary colors. The color of the filter should be the complementary to color of the solution to be measured. If a solution appears orange, this implies that orange light is not being absorbed but that the complement to orange i.e. blue is being absorbed. Therefore, the blue filter was employed for measuring absorbance of an orange solution. The use of such a filter is illustrated in Figure 6.

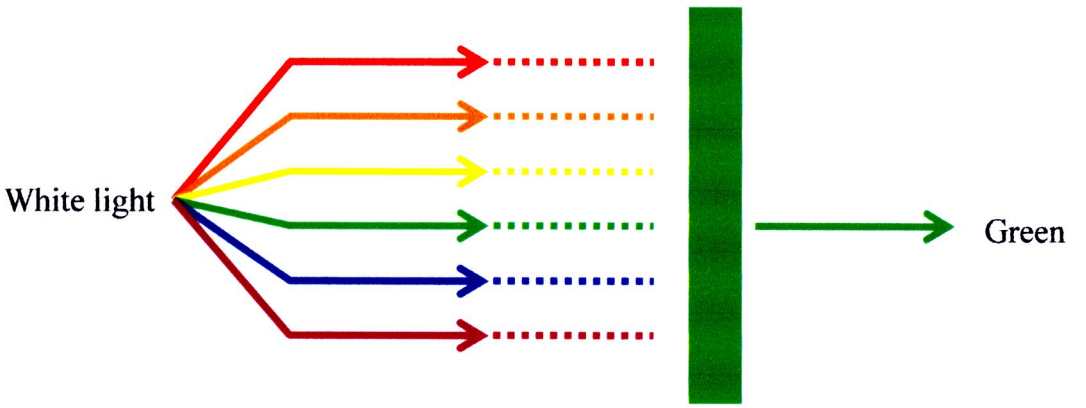


**Figure 6 Use of an absorption filter for the measurement of absorbance** [47]

Since these filters work by absorbing part of the radiation incident on them therefore they do not provide monochromatic light. A narrow band of wavelengths was given by using certain filters which actually contain two glass filters; one filter absorbs strongly above a certain wavelength, while the second absorb strongly below a certain wavelength.

The color wheel shown in Figure 7 and table of complementary colors given in Table 5 were used in filter.

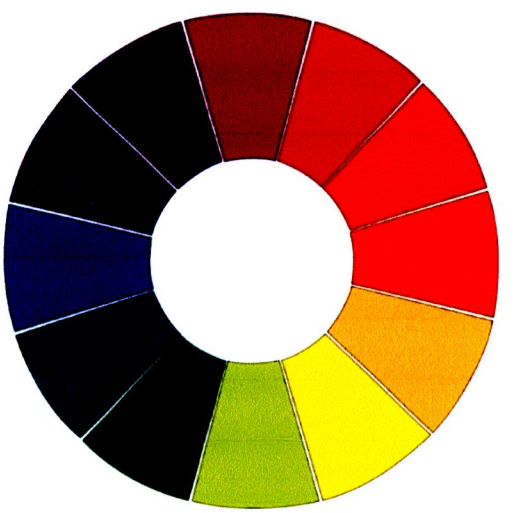

**Figure 7 The color wheel** [48]

In a color wheel, the colors, which face one another, are presented to be complementary to each other. In order to measure a red colored solution, its complementary, that is, a green colored filter should be used.

**Table 5 Complementary colors** [47]

| Wavelength region eliminated by absorption (nm) | Color absorbed | Complementary color of the residual light (as seen by the eye) |
|---|---|---|
| 400-450 | violet | yellow-green |
| 450-480 | blue | yellow |
| 480-490 | green-blue | orange |
| 490-500 | blue-green | red |
| 500-560 | green | purple |
| 560-580 | yellow-green | violet |
| 580-600 | yellow | blue |
| 600-650 | orange | green-blue |
| 650-750 | red | blue-green |

Absorption filters are simple and are totally adequate for many applications in visible range. However, interference filters was needed for extended ranges. The interference filters cover a wider range than the absorption filters. Interference filters are essentially composed of two transparent parallel films of silver, which are so close as to produce interference effects. Such interference filters are available for ultraviolet, visible and near - infrared region. The performance characteristics of interference filters are significantly superior to those of absorption (colored) filters. The effective bandwidths of these filters are narrower than absorption filters.

## 2.2 Monochromators

As mentioned earlier, monochromators are devices that can selectively provide radiation of a desired wavelength out of the range of wavelengths emitted by the source. These are of two types; the prism and grating monochromators. These are described in the following paragraphs.

### Prism monochromators

A prism disperses sunlight into seven different colors. This occurs due to the refraction of the light when it passes through the prism. The radiations of

different colors having different wavelengths are refracted to different extent due to the difference in the refractive index of glass for different wavelengths. Shorter wavelengths are refracted more than longer wavelengths as depicted in Figure 8 (A).

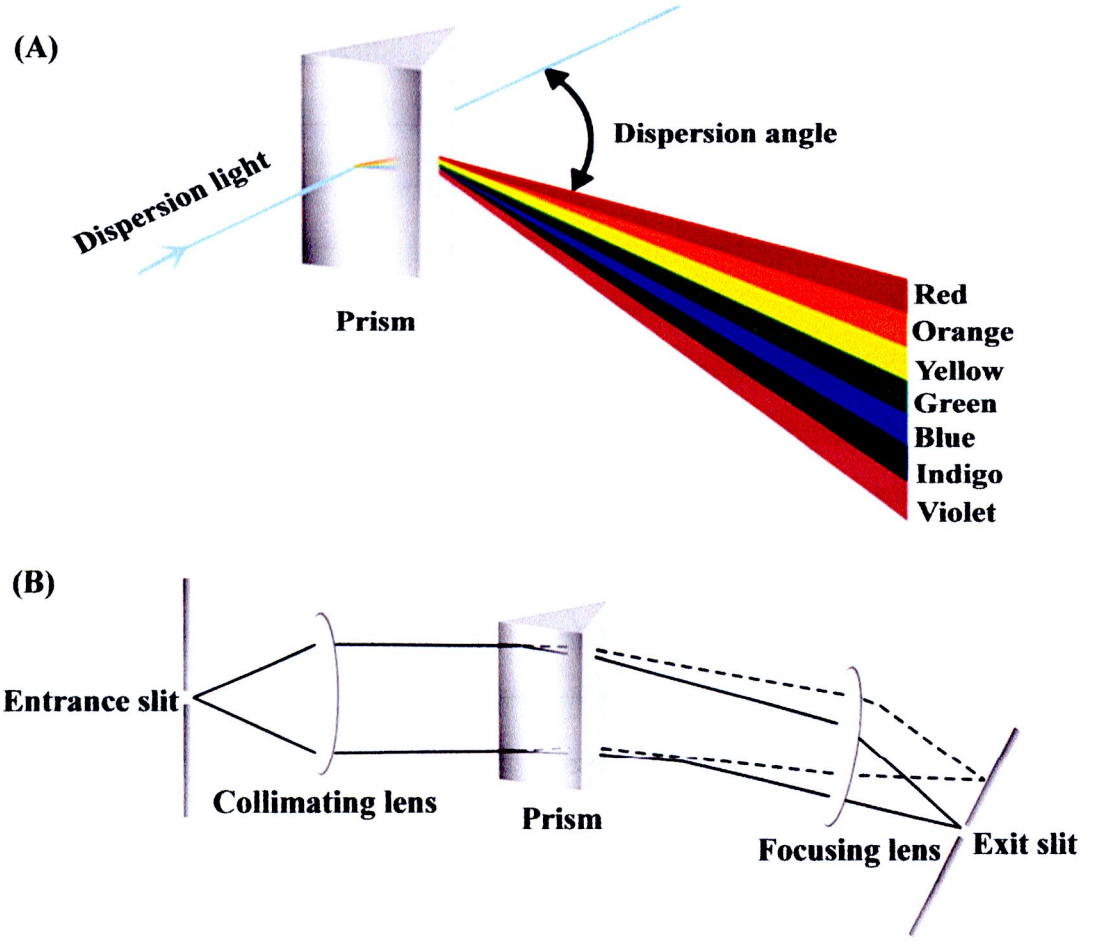


**Figure 8 Dispersion of radiation by prism (A) and schematic diagram of the prism monochromator (B)** [47]

If a prism is rotated, different wavelengths of the radiation, coming out after refracting through it, can be made to pass through the exit slit. In a prism monochromator, shown in Figure 8 (B), a fine beam of the light from the source is obtained by passing through an entrance slit. This is then collimated on the prism with the help of a lens. The refracted beams are then focused on an exit slit. The prism is then rotated in a predetermined way to provide the desired wavelength from the exit slit.

**Grating monochromators**

A grating is made by cutting or etching a series of closely spaced parallel grooves on the smooth reflective surface of a solid material as shown in Figure 8 (A). The surface is made reflective by making a thin film of aluminium on it and the etching is done with the help of a suitably shaped diamond tool. The intensity of radiation reflected by a grating varies with the wavelength, the wavelength of maximum intensity being dependent on the angle from which the radiation is reflected from the surface of the line of the grating as shown in Figure 9.

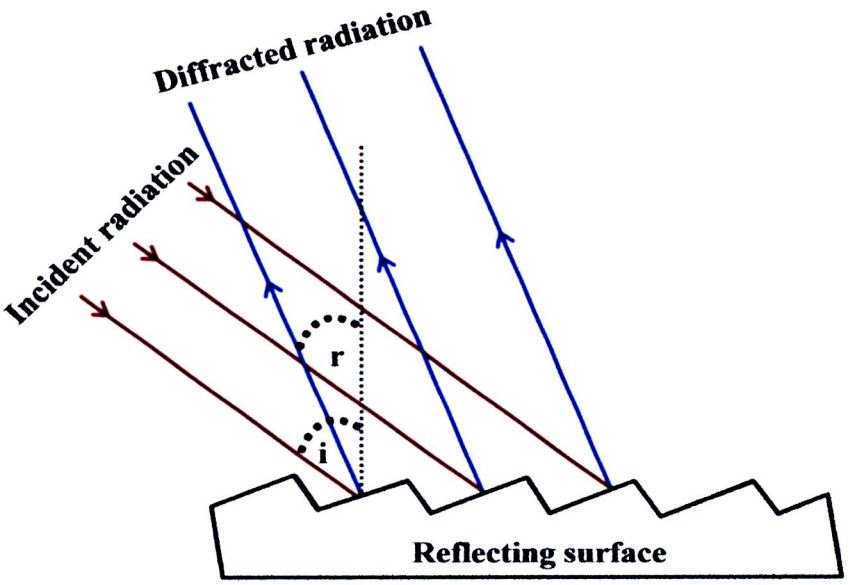


**Figure 9 Diffraction of radiation by a grating** [47]

In grating monochromator (Figure 10), a fine beam of the light from the source falls on a concave mirror through an entrance slit. This is then reflected on the grating which disperses it. The dispersed radiation is then directed to an exit slit. The range of wavelengths isolated by the monochromator is determined by the extent of dispersion by the grating and the width of the exit slit. Rotation of the grating in a predetermined way can be used to obtain the desired wavelength from the exit slit.

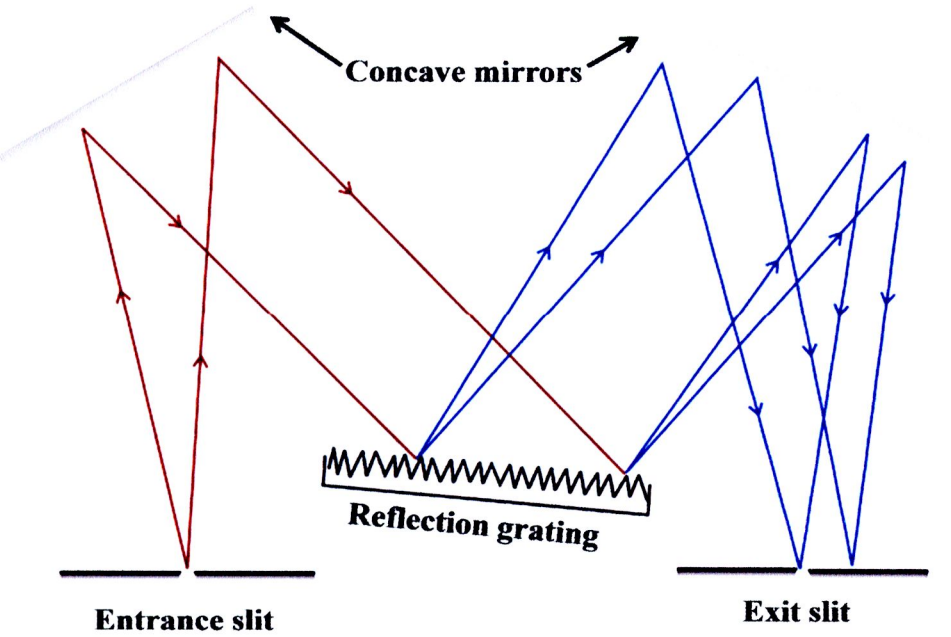


**Figure 10 Schematic diagram of a grating monochromator** [47]

**3. Sample holder** [47]

The UV-Vis absorption spectra are usually determined either in vapour phase or in solution. In order to take the UV spectrum of the analyte it is taken in a cell called a cuvette which is transparent to the wavelength of light passing through it. A variety of quartz cuvettes are available for the spectral determination in the vapour phase. These are of varying path lengths and are equipped with inlet and outlets. For measurements on solutions in the visible region the cuvettes made of glass can also be used. However, since glass absorbs the ultraviolet radiations, these cannot be used in the UV region. Therefore, most of the spectrophotometers employ quartz cuvettes (Figure 11), as these can be used for both visible and UV region. Usually square cuvettes having internal path length 1.0 cm are used, though cuvettes of much smaller path lengths say of 0.1 mm or 0.05 mm are also available.

**Figure 11 Quartz cuvette** [49]

**4. Radiation detectors** [47]

The detectors are used to convert a light signal to an electrical signal which can be suitably measured and transformed into an output. The detectors used in most of the instruments generate a signal, which is linear in transmittance i.e. they respond linearly to radiant power falling on them. The transmittance values can be changed logarithmically into absorbance units by an electrical or mechanical arrangement in the signal read out device. There are three types of detectors which are used in modern spectrophotometers. These are described in the following paragraphs.

**Phototube**

A phototube consists of a photoemissive cathode and an anode in an evacuated tube with a quartz window as shown in Figure 12 (A). These two electrodes are subjected to high voltage (about 100 V) difference. When a photon enters the tube and strikes the cathode, an electron is ejected and is attracted to the anode resulting in a flow of current which can be amplified and measured. The response of the photoemissive material is wavelength dependent and different phototubes are available for different regions of the spectrum.

**Photomultiplier tube**

A photomultiplier tube (Figure 12 (B)), consists of a series of electrodes, called dynodes. The voltage of successive electrodes is maintained 50 to 90 volt more positive than the previous one. When a radiation falls on the cathode an electron is emitted from it. This is accelerated towards the next photoemissive electrode by the potential difference between the two. Here, it releases many more secondary electrons.

These, in turn are accelerated to the next electrode where each secondary electron releases more electrons. The process continuous upto about 10 stages of amplification. The final output of the photomultiplier tube gives a much larger signal than the photocell.

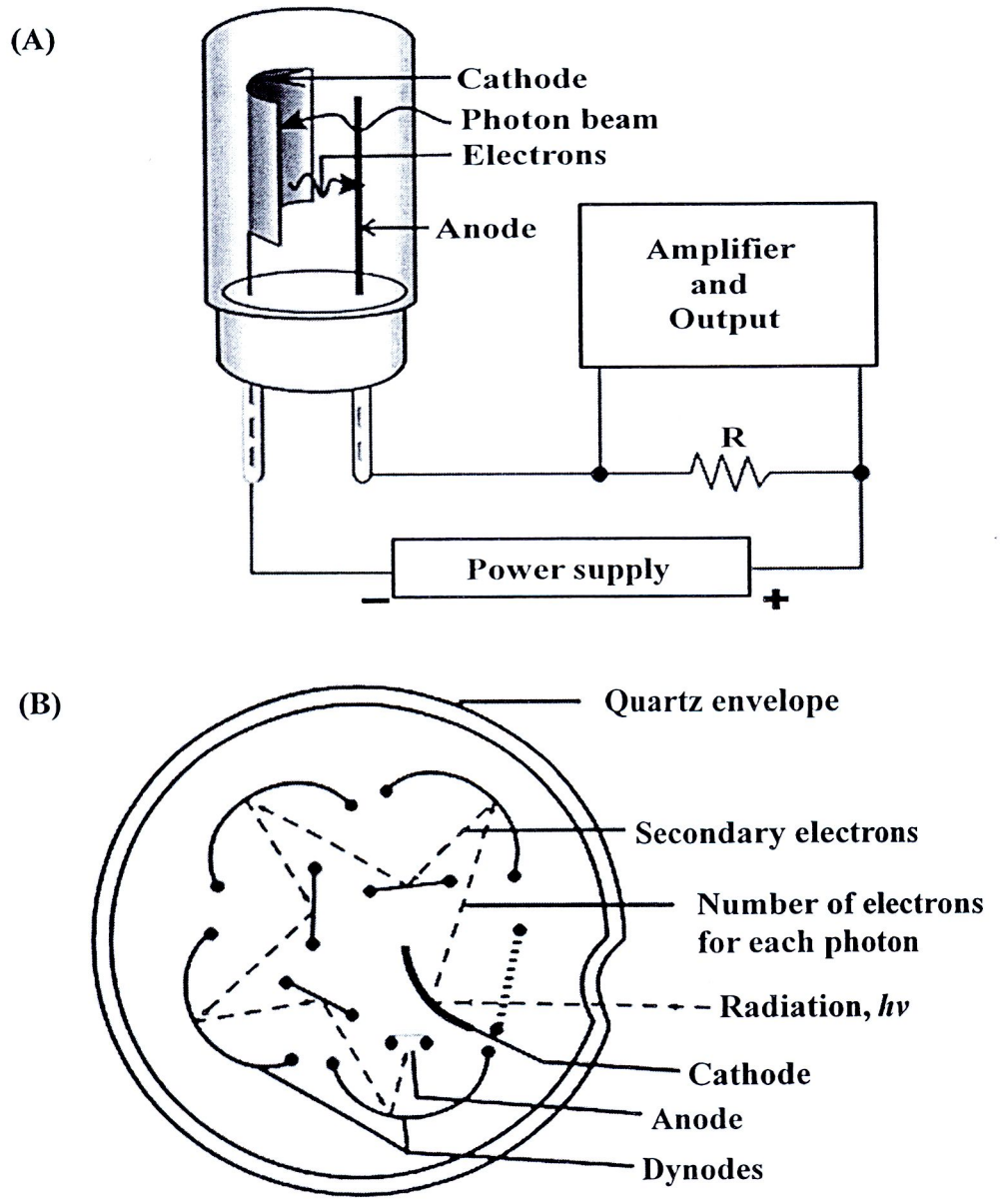


**Figure 12 Detectors of UV-Vis radiation; Phototube (A) and Photomultiplier tube (B)** [23]

**Diode array detector**

These detectors employ a large number of silicon diodes arranged side by side on a single chip. When a UV-Vis radiation falls on the diode, its conductivity increases significantly. This increase in conductivity is proportional to the intensity of the radiation and can be readily measured. Since a large number of diodes can be arranged together, the intensity at a number of wavelengths can be measured simultaneously.

Though the photodiode array is not as sensitive as the photomultiplier tube, the possibility of being able to measure a large number of wavelengths makes it a detector of choice in the modern fast instruments.

**5. Signal processing and output devices**

The electrical signal from the transducer is suitably amplified or processed before it is sent to the recorder to give an output. The subtraction of the solvent spectrum from that of the solution is done electronically. The output plot between the wavelength and the intensity of absorption is the resultant of the subtraction process and is characteristic of the absorbing species.

**Types of UV-Vis spectrophotometers** [23, 47]

Three types of spectrometers are shown in the following:

**1. Single beam spectrometers**

As the name suggests, these instruments contain a single beam of light. The same beam is used for reading the absorption of the sample as well as the reference. The schematic diagram of a typical single beam UV-Vis spectrometer is given in Figure 13. The radiation from the source is passed through a filter or a suitable monochromator to get a band or a monochromatic radiation. It is then passed through the sample (or the reference) and the transmitted radiation is detected by the photodetector. The signal so obtained is sent as a read out or is recorded.

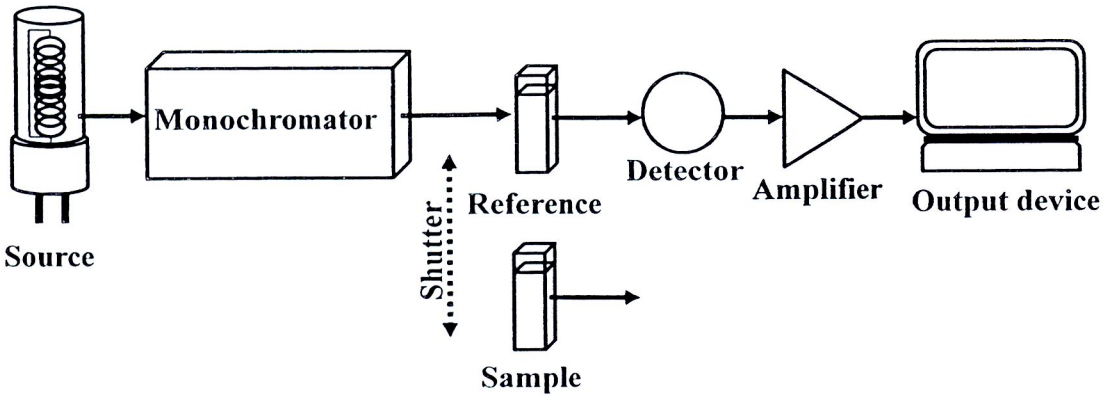


**Figure 13 Schematic diagram for a single beam UV-Vis spectrometer** [47]

Typically, two operations have to be performed, first, the cuvette is filled with the reference solution and the absorbance reading at a given wavelength or the spectrum over the desired range is recorded. Second, the cuvette is taken out and rinsed and filled with sample solution and the process is repeated. The spectrum of the sample is obtained by subtracting the spectrum of the reference from that of the sample solution.

**2. Double beam spectrometers**

In a double beam spectrometer, the radiation coming from the monochromator is split into two beams with the help of a beam splitter. These are passed simultaneously through the reference and the sample cell. The transmitted radiations are detected by the detectors and the difference in the signal at all the wavelengths is suitably amplified and sent for the output. The general arrangement of a double beam spectrometer is shown in Figure 14. There could be variations depending on the manufacturer, the wavelength regions for which the instrument is designed, the resolutions required etc.

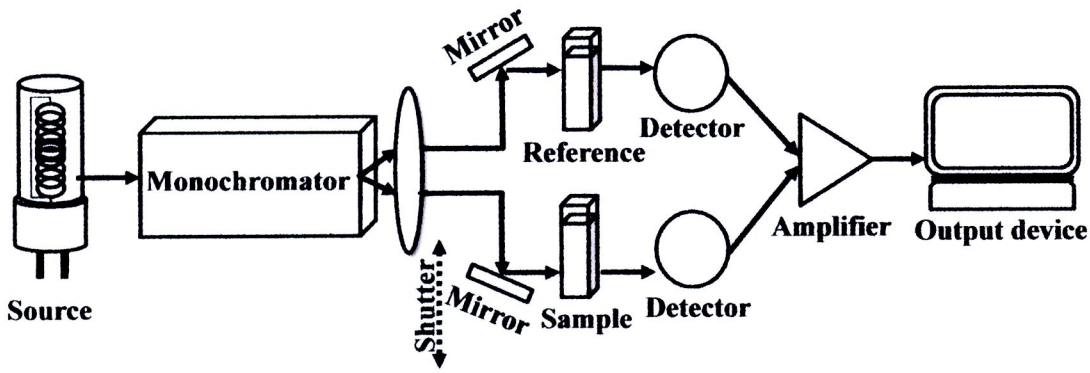


**Figure 14 Schematic diagram for a double beam spectrometer** [47]

### 3. Photodiode array spectrometer

In a photodiode array instrument, also called a multi-channel instrument, the radiation output from the source is focused directly on the sample. This allows the radiations of all the wavelengths to simultaneously fall on the sample. The radiation coming out of the sample after absorption (if any) is then made to fall on a reflection grating. The schematic arrangement of a diode array spectrometer is given in Figure 15.

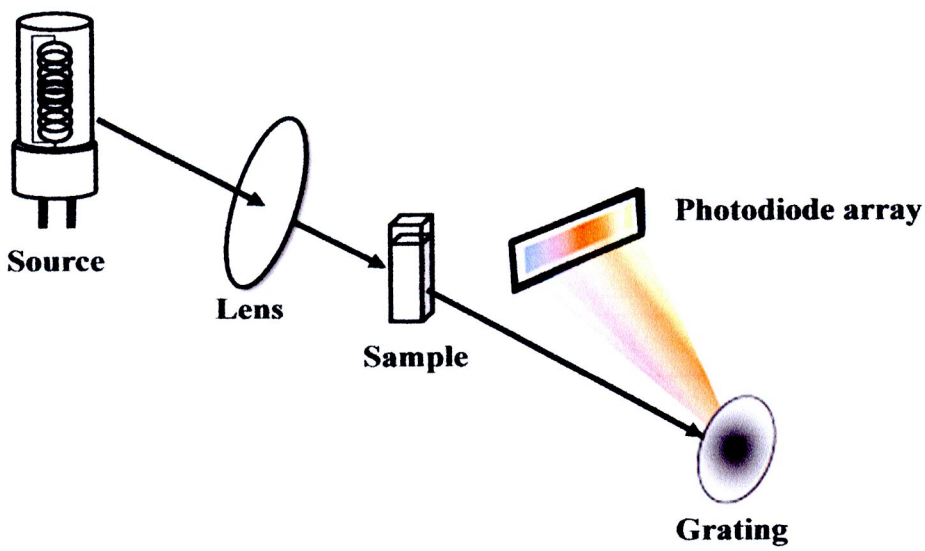


**Figure 15 Schematic diagram for a photodiode array spectrometer** [47]

The grating disperses all the wavelengths simultaneously. These then fall on the array of the photodiodes arranged side by side. In this way the intensities of all

the radiations in the range of the spectrum are measured in one go. The advantage of such instruments is that a scan of the whole range can be accomplished in a short time.

**Digital image-based colorimetry (DIC)** [50]

Until the recent advent of instrumental methods of analysis, the vast bulk of assays were performed on the basis of reagent-based colorimetric tests, either in the form of qualitative spot tests or quantitative titrations. While instrumental methods have almost completely replaced titrations, spot tests maintain their popularity as screening methods due to their ease of use and low operating cost, combined with high throughput capabilities. However, such tests have a number of well-known drawbacks such as:

1. Subjective nature of color estimations
2. Influence of background lighting
3. Impact of color blindness on the type of colors that may be used
4. Little scope for quantitative measurements
5. No compatibility with electronic report generation

Recently, there has been a rapid improvement in the technology of digital photography, both in terms of hardware and software performance. The strong indications are that this rapid development will continue over the coming years, fuelled by the need for cheap, high performance image acquisition for consumer products and the Web. The combination of digital photography and colorimetric tests potentially offers a route to high throughput qualitative and quantitative analytical measurements [50]. The analytical data that a digital camera returns are a standard trichromatic response, with 8-bit red, green and blue channels, respectively. Hence, a value is returned to the user ranging from 0 to 255 for each channel. The spectral power distribution of the light source and the spectral reflectance curve of the object being analyzed both plays an important role in the value of the color obtained through each channel [51]. Digital color varies somewhat to spectroscopic color and an understanding of this is required in order to appreciate the color signal processing techniques performed by the charge coupled device (CCD) and complementary metal oxide semiconductor (CMOS) array. By using a digital camera based on CCD and CMOS, the images obtained with high resolution have been exploited for different

purposes, such as in studies involving multivariate image regression [52], classification of inhomogeneous food matrices [53], spectral image analysis for measuring ripeness of tomatoes [54], identification of natural amino acids [55], application to determination of iron and residual chlorine in water samples [56], titration monitoring the concentration of HCl and $H_3PO_4$ in aqueous solutions and total alkalinity in mineral and tap waters [57]. However, the exploitation of digital images for the quantification of analyte concentrations is not clear in the relationship between color value and concentration of analyte, with exception of the work recently proposed by Maleki et al [58].

Maleki and coworkers [58] employed a digital camera as detection system for simultaneous determination of Al(III) and Fe(III) in alloys using the chrome azurol S (CAS) as chromogenic reagent. The RGB values associated to digital images from Al(III)-CAS and Fe(III)-CAS complexes were used in the construction of an artificial neural network (ANN) model. It should be also emphasized that due to the complex relationship between RGB values and analyte concentrations, the ANN modelling was chosen for multivariate calibration because there is no need to know the exact form of the analytical function on which the model should be built. In addition, the ANN modeling has been found in some literatures such as; prediction of leaf chlorophyll content [59], water quality measurements [60], clinical measurement of blood glucose [61], analysis of Ti in plastics [62], human serum α-fetoprotein measurement [63]. To the best of our knowledge, there was no report on the use of ANNs in combination with DIC as a detector for the determination of total phosphorus in NR latex. Therefore, in this work DIC-ANNs will be studied for the determination of total phosphorus in NR latex.

**Artificial neural networks**

One type of network sees the nodes as 'artificial neurons'. These are called artificial neural networks (ANNs) [64]. An artificial neuron is a computational model inspired in the natural neurons (Figure 16). Natural neurons receive signals through *synapses* located on the dendrites or membrane of the neuron. When the signals received are strong enough (surpass a certain *threshold*), the neuron is *activated* and

emits a signal though the *axon*. This signal might be sent to another synapse, and might activate other neurons.

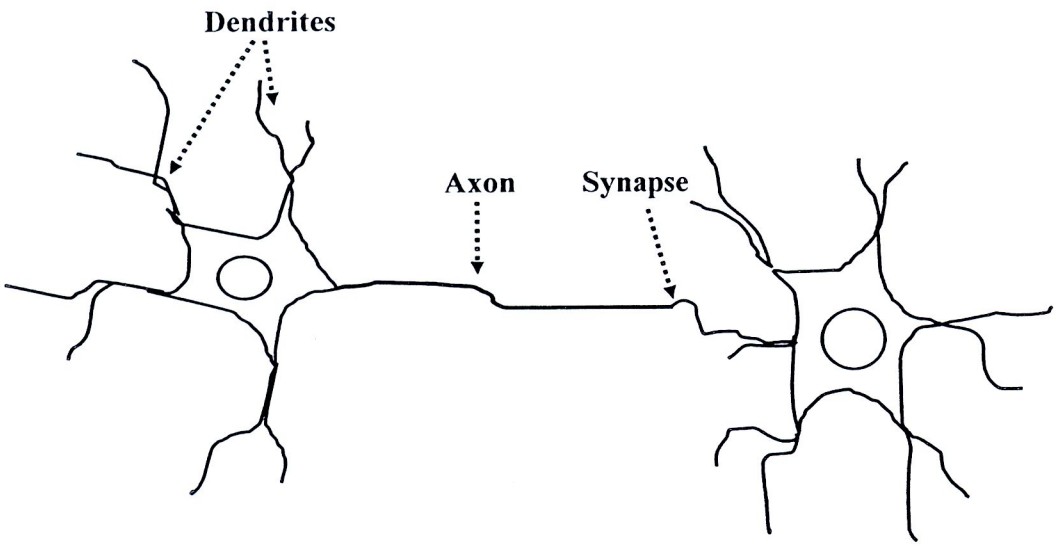


**Figure 16 Natural neurons (artist's conception)** [64]

The complexity of real neurons is highly abstracted when modeling artificial neurons (Figure 17). These basically consist of *inputs* (like synapses), which are multiplied by *weights* (strength of the respective signals), and then computed by a mathematical function which determines the *activation* of the neuron. Another function (which may be the identity) computes the *output* of the artificial neuron (sometimes in dependence of a certain *threshold*). ANNs combine artificial neurons in order to process information [65].

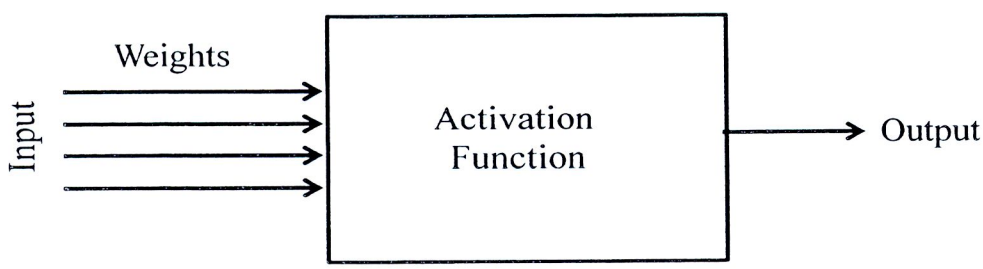


**Figure 17 An artificial neuron** [64]

The higher a weight of an artificial neuron is, the stronger the input which is multiplied by it will be. Weights can also be negative, so the signal is inhibited by the negative weight. Depending on the weights, the computation of the neuron will be different. The output could be obtained by adjusting the weights of specific inputs of an artificial neuron. But if the neurons of an ANN are hundreds or thousands, it would be quite complicated to find by hand all the necessary weights. The algorithms were found by adjusting the weights of the ANN in order to obtain the desired output from the network. This process of adjusting the weights is called learning or training. The number of types of ANNs and their uses is very high. Since the first neural model by McCulloch and Pitts [66], there have been developed hundreds of different models considered as ANNs. The differences in them might be the functions, the accepted values, the topology and the learning algorithms. Also there are many hybrid models where each neuron has more properties than the ones it is reviewing here. Because of matters of space, the ANN which learns using the back-propagation algorithm [67] was presented for learning the appropriate weights, since it is one of the most common models used in ANNs, and many others are based on it [65].

Since the function of ANNs is to process information, they are used mainly in fields related with it. There are a wide variety of ANNs that are used to model real neural networks, and study behaviour and control in animals and machines, but also there are ANNs which are used for engineering purposes, such as pattern recognition, forecasting, and data compression [68].

**Backpropagation algorithm**

The generalized delta rule [67], also known as backpropagation algorithm is explained here briefly for feed forward neural network (NN). The explanation here is intended to give an outline of the process involved in back propagation algorithm.

The NN explained here contains three layers as shown in Figure 18. These are input, hidden, and output layers. During the training phase, the training data is fed into the input layer. The data is propagated to the hidden layer and then to the output layer. This is called the *forward pass* of the backpropagation algorithm. In *forward pass*, each node in hidden layer gets input from all the nodes from input layer, which are multiplied with appropriate weights and then summed. The output of the hidden node is the non-linear transformation of this resulting sum. Similarly each node in output

layer gets input from all the nodes from hidden layer, which are multiplied with appropriate weights and then summed. The output of this node is the non-linear transformation of the resulting sum [69].

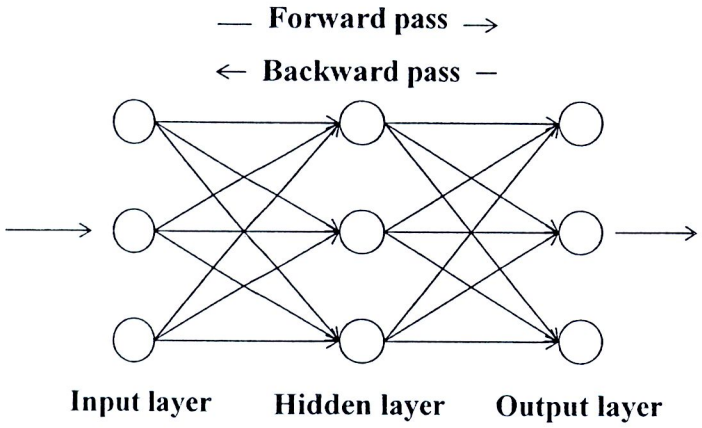


**Figure 18 Feed forward neural network** [65]

The output values of the output layer are compared with the *target output values*. The *target output values* are those that we attempt to teach our network. The error between actual output values and target output values is calculated and propagated back toward hidden layer. This is called the *backward pass* of the backpropagation algorithm. The error is used to update the connection strengths between nodes, *i.e.* weight matrices between input-hidden layers and hidden-output layers are updated. During the testing phase, no learning takes place *i.e.*, weight matrices are not changed. Each test vector is fed into the input layer. The feed forward of the testing data is similar to the feed forward of the training data [69].

In this work, a thermoreactor and UV digestion were chosen for decomposition of NR latex samples before total phosphorus determination by UV-Vis spectrophotometer compared with DIC-ANNs for which this equipment was made in our laboratory.