Chapter 1

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There is a strong correlation between the incidences of many diseases/disorders and the diet consumed by the individual. It is shown that the diet that is rich in fruits and vegetables lowers the risk of many diseases like cancer in humans. In view of this, daily food often includes nutritional rich items such as fruits like citrus, banana and grapes and sprouts like brussels, wheatgrass and of legumes. Fruits and green leafy vegetables contain nutrients such as proteins, amino acids, and dietary fiber along with essential minerals in the bioavailable form. In addition to this, they contain many useful compounds such as carotenoids, tocopherols, many polyphenolics including flavonoids and many essential chemical elements, which are known for their therapeutic, antioxidant and anti-inflammatory value. In addition, fresh green leafy vegetables like wheatgrass and spinach contain chlorophyll having potential antioxidant activity. In the last two decades, awareness about balanced diet has increased and research in nutrition and food science has focused on plant products with potential antioxidant activities. Such products are also rich in fiber, have no cholesterol and contain antioxidants such as carotenoids and flavonoids. Fresh sprouts and leafy food have become a popular part of diet. Germination/sprouting causes extensive changes in the seeds. During this stage, the synthesis of useful compounds like vitamins, amino acids and phenolics occur in the sprouts. Wheat (Triticum aestivum L.) germinated over a period of 6-10 days is known as 'wheatgrass'. Wheatgrass is consumed as it is or as an extract. The use of wheatgrass juice is also popular as ‘green food’ or ‘functional food’. In recent years, in some European countries, USA and India, wheatgrass, in the form of juice or tablet is being consumed as ‘health food’. The wheatgrass juice has been recommended for the treatment of various ailments, including chronic inflammatory conditions and malignancies. The juice is believed to be more effective when taken fresh and therefore is consumed immediately after extraction. The therapeutic properties of wheatgrass juice have been attributed to its rich nutritional content viz chlorophyll, vitamins, bioflavonoides, amino acids and the essential elements.
A focused attempt has been made in the present studies to evaluate the mineral content in the wheatgrass, determination of bioaccessibility of some essential elements present in the wheatgrass and evaluation of the antioxidant activity of fresh wheatgrass.

1.1 Essential Mineral elements:

Broadly, elements present in the living matter can be classified into three distinct groups

(i) Bulk elements

(ii) Macronutrients

(iii) Micronutrients/Essential trace elements

1.1.1 Bulk elements

These are the elements that form the bulk of a living body. Carbon, nitrogen, oxygen, hydrogen and sulphur come under this category. These elements are the major constituents of living system and hence called as building blocks. The concentration of these elements in living tissue is expressed as grams per kg of tissue weight.

1.1.2 Macronutrients:

Potassium, chlorine, phosphorus, magnesium, calcium and sodium fall under this category. They form the structural components of the living tissue and some of them like K and Na are mainly present in the body fluids (hence sometimes called as electrolytic elements) and are essential for well functioning of all cells. Their concentration and requirement in living organisms is comparatively smaller than that of bulk minerals ranging from fraction of g/kg to a few g/kg.

1.1.3 Micronutrients/essential trace elements:

Elements that are present at trace levels are called trace elements. They are sufficient for living organisms at this level. Their concentrations in living matter ranges from micrograms to fraction of milligram per kg of tissue. Their requirement for human body also ranges from a few micrograms to milligrams per day. The name ‘trace’ elements came from the fact that, such small quantities could not easily be determined by
the analytical techniques available in 20-30s of last century. The essential trace elements include iron, zinc, copper, manganese, iodine, selenium and chromium. Many elements are included in this category as the essentiality of these elements is realized over a period of last forty years due to continuous research and the advances in the modern analytical techniques and highly sophisticated instrumentation in this field. Some well accepted essential trace elements include vanadium and molybdenum. Collectively, minerals from all these three categories are called essential minerals. Essential elements are shaded in the periodic table as shown in the Fig. 1.1.

![Periodic Table](image)

Fig. 1.1 Part of the periodic table showing the essential elements

1.1.4 Essentiality of mineral elements

It is necessary to know why some of the elements are essential. The simplest definition states that the essential minerals are required for maintenance of life in terms of growth, reproduction and general well being throughout life cycle. Thus deficiency of these minerals results in departure from the normal growth and metabolism or death of the organism. Supplementation at optimal (physiological) levels leads to normal growth or prevention or cure of particular impairment caused by its deficiency. Here it should be
noted that the supplementation should be of particular mineral only, which is responsible for the observed impairment and it cannot be replaced by any other element. In the scientific world, essentiality is generally acknowledged when more than one independent investigator demonstrates it in more than one animal system.

1.1.5 Dose and response of essential elements

In the case of minerals, it is not only adequate to demonstrate the essentiality but also necessary to define its dosage and the way human body responds to it. In many cases an essential element in excess e.g. Fe, Cr etc can be harmful/toxic leading to deleterious effect on living body. The dosage of an element (including bulk element) is defined in terms of body function for which it is required. Accordingly, the response would be varying with dosage and the relation is called dose-response curve. This dose-response curve was formulated by Bertrand and is given in the Fig. 1.2. In this curve degree of health (or a particular body function) is plotted against the intake of a particular element. This dose-response curve is applicable to all the nutrients required by the body. For bulk and macronutrients dose calculation is simple as these elements are required in relatively higher amounts. On the other hand, for micronutrients, dose calculation becomes critical, as their deficiency may lead to body impairment and excess can lead to toxicity.

Fig. 1.2 Dose-response curve for essential elements
1.1.6 External supply of mineral elements

It is clear that trace minerals play a crucial role in human metabolism and are essential for general well being of the human body. Unlike proteins and enzymes, these minerals are not synthesized by the body of organism in vivo and they have to be supplied either through food (plant or animal based) or water. The supply dose depends on the age and size of the human, health condition and socio-economic background. In addition, if these minerals are supplied through diet, then dose gets altered depending on the type of diet and the conditions in which it is obtained.

In Indian scenario vegetarian habits are preferred over animal foods. Plants and plant based food products are the main source of essential minerals and other dietary components. Fruits, fresh vegetables, green leafy foods and medicinal plants gained much importance because they are not only the rich sources of essential minerals but also contain a large number of bioactive compounds with significant therapeutic activities. Both essential trace minerals and the bioactive compounds are useful for general well being of the body. For most of the common Indian population, the food they eat daily is the main source of minerals. These include legumes, cereals, vegetables and fruits.

1.1.7 Need for Elemental Analysis of food and food products

Elements are widely distributed in the earth’s crust. About 88 elements in the periodic table are naturally occurring and others are synthetic. The elements like Si, Al, Ca, Mg, Fe, O, Ti, Na and K are the major components accounting for 99% by weight of earth’s crust. Human food contains plant based items and/or animal products. Most of the animals get their food through plants. Therefore human food chain is linked to plants which get minerals from soil and water. To understand the utility of food items, it is imperative to determine the mineral elements and their compounds in various food and animal products, soil and water. A reliable analytical method is essential to estimate the chemical elements in the plant and animal food products. The chosen method could estimate them accurately and reliably. With the advances made in the sophisticated analytical instrumentation and associated softwares, it is possible to obtain the analytical information that is highly reliable.
1.1.8 Analytical Methods for Trace Elemental Analysis

1.1.8.1 General methods

Some important steps in the analysis of elements present in the sample are sample collection, subsampling, choosing an optimum method, analysis and interpretation of analytical data. A large number of methods have been developed over the years for trace element analysis with varying detection ability ranging from ppm (µg g⁻¹) to ppb (ng g⁻¹) levels. Choice of the method depends on many factors such as complexity of the samples, analyte concentration, homogeneity of the analyte in the sample, method of analysis, expertise available to the analytical laboratory and good laboratory practices. A few methods often used by an analytical chemist are:

1. Spectroscopic methods
2. Electrochemical methods
3. Radioanalytical methods
4. Spectrophotometric methods

Atomic Absorption Spectrometry (AAS), Atomic Fluorescence Spectrometry (AFS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) are some of the spectroscopic methods. In these methods intensity of absorbed/emitted light is measured to find out the concentration of element of interest. The electrochemical methods include electrogravimetry, voltametry, coulometry, potentiometry and polarography. In electrochemical methods concentration of an element is determined by measuring electrochemical signal like voltage, current, charge or pH of the sample solution containing the analyte. The nuclear analytical methods include techniques like Activation Analysis (AA), Particle Induced X-ray/Gamma-ray Emission (PIXE/PIGE), Nuclear Reaction Analysis (NRA) and X-ray Fluorescence (XRF) spectrometry. An isotopic signal like intensity of a γ-ray or an elemental signal like intensity of X-rays is measured using nuclear instrumentation which is related to concentration of the element/isotope. In the case of spectrophotometric methods, absorption of light in ultraviolet or visible region is measured after developing colour by chemical treatment to find the concentration of the analyte. Almost all the elements can be measured by spectrophotometric methods.
1.1.8.2 Radio-analytical Methods

Analytical techniques such as tracer techniques, isotope dilution, radiometric titration and activation analysis are some of the nuclear analytical techniques. George de Hevesy was the first to use radioisotopes as tracers. He also discovered activation analysis. This branch dealing with analytical techniques is distinguished as ‘Radio-analytical chemistry’. Discovery of neutron, artificial radioactivity and nuclear fission, and the construction of nuclear reactors and particle accelerators in the period of 1930 to 1950, nuclear analytical methods became powerful tools of analysis and found applications in many areas of research. The use of semiconductors as radiation detectors, which began in the 60’s brought in a new dimension in the identification and accurate assay of individual radionuclides from a complex mixture. Fig. 1.3 depicts some of the important nuclear analytical techniques. Of all the activation analysis methods, Neutron Activation Analysis (NAA) is the most widely used for routine analysis owing to its low detection limits, higher accuracy and precision for many elements and simultaneous multielement analysis capability. During last 60 years, it has reached the status of an independent analytical tool with many interdisciplinary applications. It is used as a reference method for elemental analysis in various matrices.
Fig 1.3 A general survey of Radio-analytical techniques.
1.1.9 Neutron Activation Analysis

1.1.9.1 Introduction

Neutron activation analysis (NAA) is an important technique in analytical chemistry. With the availability of high flux nuclear reactors and rapid developments in the sophisticated instrumentation and the advent of high purity germanium (HPGe) detectors with high resolution, the technique has evolved as a powerful tool for analysis. The technique is highly selective, sensitive and non-destructive with simultaneous multielement analysis capability. Extensive applications of NAA in the fields of biology, geology, health science, environmental science, material science, forensic science and nuclear technology for determination of various elements at trace levels are well documented.

Enrico Fermi, for the first time reported that the radioactive product could be produced by neutron activation\(^\text{45}\). Prof. Georg de Havesy and Hilde Levi, in 1936 were the first to determine dysprosium impurity at 0.1% level in yttrium samples by irradiating the samples with neutrons obtained from radium-beryllium source\(^\text{41}\). This was the first work where neutrons were used for chemical analysis. This has demonstrated the utility and capability of neutrons that can be exploited in the analytical chemistry. G.T. Seaborg and J.J. Livingwood in 1938 showed the non-destructive aspect of activation analysis in general\(^\text{46}\). Clark and Overman\(^\text{47}\) gave the first systematic presentation of neutron activation analysis in 1947. Liddicotte and Raynolds\(^\text{48}\) had subsequently put the technique on routine use in 1951. Construction of nuclear reactors that provide high neutron flux in the 60’s of 20\(^{\text{th}}\) century gave an impetus to NAA to become a powerful tool for multielement determination. With the availability of high flux reactors in conjunction with state-of-art nuclear instrumentation for radiation detection, activation analysis gained a wide popularity as a reference method for the determination of almost 80 elements in the periodic table.

1.1.9.2 Principle

Neutron, being a non-charged particle (i.e. zero columbic barrier) interacts with the nuclei of all isotopes, causing nuclear reactions. The product formed in such a nuclear
reaction, often is a radioisotope. By measuring the induced radioactivity, the isotopic concentration of the element undergoing nuclear reaction can be calculated, which is used to calculate chemical concentration. This is the general principle of neutron activation analysis. Fig. 1.4 shows the schematic representation of NAA.

When a target $^A_ZX$ is bombarded with neutrons, a compound nucleus $^{A+1}_ZX^*$ is formed in excited state that decays to ground state by emitting gamma rays which are called prompt gamma rays. Prompt $\gamma$-rays are measured to analyze the sample and the technique is called prompt gamma neutron activation analysis (PGNAA). Compound nucleus in the ground state $^{A+1}_ZX$ decays by emitting a $\beta^-$ particle. The product formed after emitting a $\beta^-$ particle is an isotope of another element $^{A+1}_{Z+1}Y$ that de-excites to ground state (Fig. 1.4) by emitting characteristic gamma rays. These are called delayed gamma rays and are measured in conventional neutron activation analysis (NAA). Intensity of these gamma rays are related to isotopic concentration of $^{A+1}Y$ and in turn to isotopic concentration of the analyte $^{A+1}_ZX$. Elemental concentration is obtained from isotopic concentration.
1.1.9.3 Activity formed in neutron activation

In neutron induced reaction of the type \(^{A}X\) \((n,\gamma)\) \(^{A+1}X\), activity \((A)\) formed is given by,

\[
A = \left( \frac{N_A \theta w}{M} \right) \sigma \phi S D \quad \text{.................. (1.1)}
\]

where,

\(- N_A = \text{Avogadro's number} \)
\(- \theta = \text{Isotopic abundance of analyte element, \%} \)
\(- w = \text{weight of the sample, g} \)
\(- M = \text{Atomic mass of the element} \)
\(- \sigma = \text{neutron capture cross section of the isotope, cm}^2 \)
\(- \phi = \text{neutron flux, n cm}^{-2} \text{s}^{-1} \)
\(- S = \left[ 1 - e^{-\lambda t} \right] = \text{Saturation Factor} \)
\(- D = e^{-\lambda t'} = \text{Decay factor} \)
\(- \lambda = \text{decay constant, t= duration of irradiation and t'= Cooling period} \)

Radioactivity of the product \((A)\) is measured by \(\gamma\)-ray spectrometry using High Purity Germanium (HPGe) detector and the peak area, \(P_A\) corresponding to a characteristic gamma ray is related to radioactivity by equation 1.2.

\[
P_A = \frac{N_A \theta w}{M} \sigma \phi S D C E \gamma \quad \text{.................. (1.2)}
\]

where, \(C = (1 - e^{-\lambda t CL} / \lambda)\) is the correction factor for decay during counting period of \(CL\), \(\varepsilon\) is the absolute full-energy peak detection efficiency and \(\gamma\) is the gamma-ray abundance. From the measured activity using the values of neutron flux, cross section, time of irradiation, half life, gamma ray abundance and detector efficiency the quantity of the element \((w)\) can be calculated.

1.1.9.4 Neutron Sources

The basic requirement to perform neutron activation analysis is the availability of a suitable neutron source. Table 1.1 lists the variety of commonly used neutron sources\(^{44}\).
Three types of sources are generally utilized viz. isotopic sources, accelerator based sources and nuclear reactors. Nuclear reactors are the best neutron sources where highest neutron flux in the range of $10^{11}$-$10^{15}$ n cm$^{-2}$ $s^{-1}$ is available.

### Table 1.1: Neutron sources

<table>
<thead>
<tr>
<th>Source*</th>
<th>Average Neutron Energy (MeV)</th>
<th>Neutron Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Photonuclear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{88}$Y + Be (106.6 d)</td>
<td>0.16</td>
<td>$1.0 \times 10^5$ n s$^{-1}$ Ci$^{-1}$</td>
</tr>
<tr>
<td>$^{124}$Sb + Be (60.2 d)</td>
<td>0.02</td>
<td>$1.9 \times 10^5$ n s$^{-1}$ Ci$^{-1}$</td>
</tr>
<tr>
<td>II. Alpha emitters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{239}$Pu + Be (2.4 x 10$^4$ y)</td>
<td>3-5</td>
<td>$\sim 10^7$ n s$^{-1}$ Ci$^{-1}$</td>
</tr>
<tr>
<td>$^{226}$Ra + Be (1600 y)</td>
<td>3.6</td>
<td>$1.1 \times 10^7$ n s$^{-1}$ Ci$^{-1}$</td>
</tr>
<tr>
<td>$^{241}$Am + Be (433 y)</td>
<td>3-5</td>
<td>$2.2 \times 10^6$ n s$^{-1}$ Ci$^{-1}$</td>
</tr>
<tr>
<td>III. Spontaneous fission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{252}$Cf (2.64 y)</td>
<td>2.3</td>
<td>$2.3 \times 10^9$ n s$^{-1}$ mg$^{-1}$</td>
</tr>
<tr>
<td>IV. Accelerators</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^3$H(d, n)$^4$He</td>
<td>14</td>
<td>$10^8$ - $10^{11}$ n s$^{-1}$</td>
</tr>
<tr>
<td>V. Cyclotron</td>
<td>Broad range</td>
<td>$2 \times 10^{11}$ n s$^{-1}$</td>
</tr>
<tr>
<td>VI. Nuclear Reactor</td>
<td>1.2</td>
<td>$10^{11}$-$10^{15}$ n cm$^{-2}$ s$^{-1}$</td>
</tr>
</tbody>
</table>

*Half life of the isotope is given in the parentheses

### 1.1.9.5 Neutron spectrum in Nuclear Reactors

Neutrons produced in a reactor are not monoenergetic. The energy of neutrons is in the range of eV to about ten MeV. According to energy of neutrons, they are classified as cold, thermal, epithermal, intermediate, and fast neutrons as given in Table 1.2. A typical neutron spectrum$^{44}$ in reactor along the energy regions is shown in the Fig. 1.5. Neutrons with different energies can induce different types of nuclear reactions such as $(n, \gamma)$, $(n, n)$, $(n, p)$, $(n, 2n)$ and $(n, \alpha)$. The $(n, \gamma)$ reaction is most commonly used reaction in NAA. An irradiation position in a reactor having higher thermal to epithermal neutron flux ratio ($\phi_t/\phi_e$) is the preferred choice for activation analysis.
### Table 1.2: Classification of neutrons with the energy range

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Type of Neutron</th>
<th>Energy Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cold</td>
<td>0.003-0.015 eV</td>
</tr>
<tr>
<td>2.</td>
<td>Thermal</td>
<td>0.0252 eV - 0.55 eV</td>
</tr>
<tr>
<td>3.</td>
<td>Epithermal</td>
<td>0.2-1 eV</td>
</tr>
<tr>
<td>4.</td>
<td>Resonance</td>
<td>1 eV - 500 keV</td>
</tr>
<tr>
<td>5.</td>
<td>Fast</td>
<td>&gt; 500 keV</td>
</tr>
</tbody>
</table>

Fig. 1.5 The neutron spectrum in a reactor irradiation facility. The neutron flux is given for 1 MW reactor power.

#### 1.1.9.6 Nuclear Reaction

Nuclear reaction probability is called nuclear cross-section. Its symbol is $\sigma$ and unit is cm$^2$. Reaction cross section varies with energy of the projectile e.g. neutron. For neutron induced reactions, generally reaction cross section decreases with increasing energy barring some resonance peaks. Fig. 1.6 shows the energy dependence of cross section ($\sigma$) for neutron induced reaction of $^{197}$Au$^{44}$. 
1.1.9.7 Methodologies in NAA

Different methodologies of NAA are: (i) energy dependent NAA, (ii) standardization methods in NAA and (iii) different approaches in NAA.

1.1.9.7.1 Energy dependant NAA

Depending on the energy of the neutrons utilized following methods are developed-

(a) Thermal Neutron Activation Analysis (TNAA)
(b) Epithermal Neutron Activation Analysis (ENAA)
(c) Fast Neutron Activation Analysis (FNAA)

a) Thermal Neutron Activation Analysis (TNAA):

In the thermal neutron activation analysis, neutrons of energy upto 0-0.55 eV are used. The average energy of the thermal neutrons in a nuclear reactor is approximately 0.0253 eV. The most common reaction caused by thermal neutrons is of \((n, \gamma)\) type. Thermal neutron flux component in a reactor depends on the extent of moderation, irradiation position and type of the reactor. In E8 position of Apsara reactor, the thermal
neutron component is 98%. In the PCF position of Dhruva reactor, it is >99%. Most of the elements in periodic table are analyzed using thermal neutrons with good sensitivity.

b) Epithermal Neutron Activation Analysis (ENAA):

Activation Analysis using epithermal and resonance neutrons energy ranging from 0.55 eV (cadmium cut-off energy) onwards is known as Epithermal Neutron Activation Analysis (ENAA). In the broadly defined epithermal or more accurately epi-cadmium energy region the neutrons do not follow the 1/v variation. For elements with large resonance peaks (Fig. 1.6), ENAA is a very sensitive and selective technique. In ENAA, neutrons of low energy are filtered off using suitable filters. A cylindrical box of cadmium (0.55 eV cut-off) or a boron carbide box (cut-off energy 100-300 eV) are used as filters. It also helps in reducing the activation of those elements having higher capture cross-section with thermal neutrons. For example, in biological samples, significant levels of elements such as Na, Al, K, Sc, Cr, Mn, Fe and La are present. Most of the isotopes of these elements have higher $\sigma_{th}$ (thermal neutron capture cross section). Formation of activation products of these elements is suppressed, and hence spectral interferences are minimized. If a low-level analyte has a large resonance integral, irradiation of the sample with epithermal neutrons will lead to reliable results with better limit of detection. ENAA is mostly used for geological and biological samples to suppress the high thermal neutron induced activities from matrix elements. Elements with high resonance integral to thermal neutron capture cross section ratios ($I_o / \sigma_{th} = Q_o / Q_{th}$) are usually analyzed by ENAA. Such elements are Ag, As, Au, Ba, Br, Cd, Cs, Ga, Gd, In, Mo, Ni, Pd, Pt, Rb, Sb, Sc, Sm, Sr, Ta, Tb, W, Th and U.

c) Fast Neutron Activation Analysis:

Neutrons with energies, from 0.5 MeV and above are conventionally called fast neutrons. In this energy region, $(n,\gamma)$ reactions are competed by the threshold reactions e.g. $(n, n')$, $(n, p)$, $(n, \alpha)$ and $(n, 2n)$ reactions. A common and particularly useful form of FNAA is based on reactions with 14 MeV neutrons that are produced by $(d,t)$ accelerators known as neutron generators. The FNAA is not as sensitive as TNAA because of the lower cross sections and smaller fluxes of fast neutrons. However, FNAA
is capable of determining many of the light elements that cannot be done with either thermal or epithermal NAA. The common elements that are determined by FNAA are O, N, F, Mg, Si, P, Fe, Cu, Zn, Zr, Th and U. Some of the nuclear reactions and their associated decay data are listed in Table 1.3.

Table 1.3: Nuclear data for some fast neutron induced reactions.

<table>
<thead>
<tr>
<th>Element</th>
<th>Nuclear reaction</th>
<th>Product Half-life</th>
<th>Energy of the Gamma-ray/keV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(a) Gamma ray measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>$^{16}\text{O} (n, p) \ ^{16}\text{N}$</td>
<td>7.13 s</td>
<td>6130, 7120</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>$^{14}\text{N} (n, 2n) \ ^{13}\text{N}$</td>
<td>9.97 min</td>
<td>511</td>
</tr>
<tr>
<td>Fluorine</td>
<td>$^{20}\text{F} (n, p) \ ^{19}\text{O}$</td>
<td>30 s</td>
<td>197.4</td>
</tr>
<tr>
<td>Magnesium</td>
<td>$^{24}\text{Mg} (n, p) \ ^{24}\text{Na}$</td>
<td>15 h</td>
<td>1368.5</td>
</tr>
<tr>
<td>Silicon</td>
<td>$^{28}\text{Si} (n, p) \ ^{28}\text{Al}$</td>
<td>2.24 min</td>
<td>1779</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>$^{31}\text{P} (n, \alpha) \ ^{28}\text{Al}$</td>
<td>2.24 min</td>
<td>1779</td>
</tr>
<tr>
<td>Iron</td>
<td>$^{56}\text{Fe} (n, p) \ ^{56}\text{Mn}$</td>
<td>2.58 h</td>
<td>847</td>
</tr>
<tr>
<td>Copper</td>
<td>$^{63}\text{Cu} (n, 2n) \ ^{62}\text{Cu}$</td>
<td>10 min</td>
<td>511</td>
</tr>
<tr>
<td>Zinc</td>
<td>$^{64}\text{Zn} (n, p) \ ^{64}\text{Cu}$</td>
<td>12.8 h</td>
<td>511</td>
</tr>
<tr>
<td>Zirconium</td>
<td>$^{90}\text{Zr} (n, 2n) \ ^{89m}\text{Zr}$</td>
<td>4.18 min</td>
<td>588</td>
</tr>
<tr>
<td><strong>(b) Delayed neutron measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thorium</td>
<td>Fission</td>
<td>-</td>
<td>Delayed neutron</td>
</tr>
<tr>
<td>Uranium</td>
<td>Fission</td>
<td>-</td>
<td>Delayed neutron</td>
</tr>
</tbody>
</table>

1.1.9.8 Standardization methods in NAA

The activities produced in neutron activation can be used to determine the analyte concentration. Following three methods of NAA are being used.

1. Absolute method
2. Relative method
3. The $k_0$-standardization method
1.1.9.8.1 Absolute method

In the absolute method, elemental concentrations are calculated by using the data for all the parameters in equation 1.3. This method was developed by Girardi et al.\textsuperscript{19} in the 60s. They have determined 13 elements using a calibrated $\gamma$-ray spectrometer for measuring the absolute activity.

$$w(g) = \frac{P_A}{SDC} \cdot \frac{M}{N_A \theta \gamma (\sigma \phi)} \cdot \frac{1}{\epsilon}$$

In this method quantities like $P_A$, $\phi$ and the absolute detection efficiency ($\epsilon$) are experimentally determined and capture cross section ($\sigma$), gamma-ray abundance ($\gamma$) and other nuclear data are taken from literature. The peak areas ($P_A$) are evaluated from measured gamma spectra using a suitable peak-fit PC based software. Neutrons obtained in a reactor are not monoenergetic (Fig. 1.5) In addition $\sigma$ varies with the energy of neutrons (Figs 1.5 and 1.6), it is difficult to obtain the exact value of these two. Accordingly the uncertainties would be larger to the extent of 20% or more. In addition, determination of various parameters is time consuming and difficult to carry out for each element if multielement analysis is to be performed.

1.1.9.8.2 Relative Method:

This method is most commonly employed in many radioanalytical laboratories. In this method, samples and the standards are co-irradiated in a nuclear reactor. The induced radioactivity of sample and standard is assayed under identical geometrical conditions. The peak areas are corrected for decay during cooling period for both samples and standards, and concentrations are calculated by comparing the activities of the sample to that of standard. Concentration of an analyte in the sample is calculated using eq. 1.4

$$W_{samp} = W_{std} \cdot \frac{A_{samp} \cdot e^{-\lambda t_{samp}}}{A_{std} \cdot e^{-\lambda t_{std}}} \cdot \epsilon$$

In this method the concentrations in the complex samples can be evaluated with better precision and accuracy compared to the absolute method.

One disadvantage of the method is that, it is essential for the analyst to have an a priori knowledge of the analytes in the samples. This may be very difficult for the
complex matrix such as of biological and geological origin. In such cases one has to use the standards, which have same or similar matrix composition as that of the sample. Now-a-days standard reference materials (SRMs) and certified reference materials (CRMs) are readily available from international agencies such as National Institute of Standards and Technology (NIST), International Atomic Energy Agency (IAEA) and United States Geological Survey (USGS). These CRMs/SRMs can also be used as control samples to evaluate the accuracy of the method. However, the possibility of obtaining standards with identical composition is difficult. Additional problem could be inhomogeneity in the neutron flux at the irradiation position due to neutron attenuation inside the matrix. It is possible that samples and standards in the irradiation container may receive different fluxes.

1.1.9.8.3 Single Comparator Method (k₀-NAA)

Single comparator method or k₀-NAA method, is based on co-irradiation of the sample and of a neutron flux monitor, and on using an experimentally determined composite nuclear constant k₀. The analysis results are linked to k₀ factors, absolute detection efficiency of the detector and neutron spectrum characteristics. Concentration of an element is calculated using equation 1.5.

\[ C_i (\mu g.g^{-1}) = \frac{P_a}{S.D.C.W} \cdot \frac{1}{k_{0,exp}} \cdot \frac{f + Q_0(\alpha)^*}{f + Q_0(\alpha)} \cdot \frac{\varepsilon^*}{\varepsilon} \quad (1.5) \]

The symbol "*" refers to the parameters of the comparator.

As the factor k₀ is used for the calculation of concentration, the method is referred as k₀ NAA. The accuracy and consistency of the nuclear data play a significant role in the standardization of k₀-NAA. Out of these nuclear constants, data on cross-sections and gamma ray emission probabilities are sometimes reported with large uncertainties.

1.1.9.9 Different approaches in NAA

There are mainly six different approaches of NAA, which are used depending on the matrix and elements to be determined.
1.1.9.9.1 Instrumental Neutron Activation Analysis (INAA)

In the INAA, a sample is irradiated without any pre-chemical treatment and the radioactivity produced is directly measured using a suitable radiation detector. Radioactivity measurement is generally carried out by high-resolution gamma ray spectrometry using high purity germanium (HPGe) detector. In multi-element analysis, the activation products are identified by their characteristic gamma lines and half lives. By adjusting irradiation and cooling times more than 70 elements can be analyzed in a set of experiments. The irradiation periods chosen are of three types; short, medium and long, depending on the half-lives of activation products to produce sufficient activity. For short irradiations of 30 to 300 seconds duration, a Pneumatic Carrier Facility (PCF) is used for irradiations and radioactive assay of short-lived activation products (half-lives 1-10 minutes) is carried out. Longer irradiation times (hours to days) and longer measurement times facilitate the determination of elements having medium and long-lived activation products.

This method is most preferred because there is no reagent blank. Sample handling before and after irradiation is minimal in INAA.

1.1.9.9.2 Radiochemical Neutron Activation Analysis (RNAA)

In Radiochemical NAA, the chemical separations on the sample are carried out after irradiation to achieve selectivity by reducing spectral interferences. These chemical procedures may separate single element or group of elements. The separated fractions are then assayed for the analyte activity. RNAA is generally used for samples with complex matrix such as geological and biological origin. In biological samples, the
elements As, Cd, Cu, Hg, Mo, Se and Zn are often required to be determined which are generally present at low levels. Activities of these elements produced are masked by the activities of elements such as Na, K and Br present in the bulk. Therefore their determination at trace level is very difficult. In such cases, chemical isolation of the required analyte enables its estimation. In the RNAA procedure inactive reagents are added and hence there is no contamination to the active analyte and does not influence the measurements adversely. Different separation methods, including solvent extraction, precipitation and ion exchange procedures are employed in RNAA. The separated fractions are assayed by gamma-ray spectrometry in HPGe detectors or NaI(Tl) detector depending on complexity of the spectrum. It is advisable to validate the method using biological RMs.

1.1.9.9.3 Chemical Neutron Activation Analysis (CNAA)

If chemical separations are employed before sample irradiation then it is called CNAA\(^{35,44}\). In this procedure the analyte is chemically separated from the sample solution and then subjected to irradiation. Chemical isolation and preconcentration are achieved in CNAA. Sensitivity is enhanced in CNAA, since large samples can be taken for processing as compared to RNAA and the major matrix elements are removed in preconcentration step. Low background and higher analyte signals are achieved leading to better sensitivity. As an example, if the sample contains uranium, CNAA is preferred as uranium can be removed by chemical separation before irradiation. Otherwise, presence of uranium causes interference at \(^{140}\)La, \(^{141,143}\)Ce and \(^{147}\)Nd activities due to neutron induced nuclear fission of uranium and at \(^{153}\)Sm due to the gamma spectral interference from \(^{239}\)Np formed as an activation product of \(^{238}\)U. The gamma lines from \(^{239}\)Np also complicate the gamma ray spectrum in the low energy region. The limitation in this procedure is that the reagent blanks might be present and one has to ensure that no loss of analyte occurs during chemical separation.

1.1.9.9.4 Derivative Activation Analysis (DAA)

Derivative activation analysis (DAA) in conventional NAA is a novel analytical approach that is used for elements having very low sensitivity e.g.; Li, Be, Ni, P, Nb, Rh,
Si, Sn, Tl, Pb and Bi in conventional NAA. In DAA the element to be determined is chemically combined with an other element known as surrogate element, which has high sensitivity for NAA. The compound or complex formed is chemically separated from the rest of the matrix and neutron irradiated. After the irradiation the activity of the surrogate element is assayed and with the knowledge of stoichiometry of the compound/complex formed, amount of the analyte is evaluated. The choice of the surrogate element is such that it should have superior properties for NAA like high isotopic abundance, higher thermal neutron cross-section, product nuclide with suitable half-life and intense interference free gamma lines. Phosphorus was estimated with molybdenum as surrogate element by separating and neutron irradiating phosphomolybdic complex.

1.1.9.9.5 Cyclic Activation Analysis (CAA)

Cyclic activation analysis (CAA) is mainly for the determination of short-lived activation products by use of repetitive short irradiation and counting periods and summing of the γ-ray spectra obtained. This procedure enhances counting statistics for the short lived species and thereby increases the effective signal-to-noise ratio in the determination of short half life radionuclides with respect to interfering long lived radionuclides. Both thermal and 14-MeV neutrons are used in CAA. In each short irradiation, saturation activity of the short-lived nuclides can be obtained. The total detector response (DR) for a short-lived indicator radionuclide determined by cyclic activation analysis (CAA) increases linearly with experiment time (irradiation time + counting time).

1.1.9.9.6 Prompt Gamma-ray Neutron Activation Analysis (PGNAA)

Prompt Gamma-ray Neutron Activation Analysis (PGNAA) is a complementary technique to conventional NAA method and is capable of analyzing elements like H, B, C, N, P, Si, S, Cl, Ti, Co, Cd, Gd, Sm and Eu. It is an online measurement technique. Neutrons from a cold or thermal neutron source are used in the prompt gamma-ray activation system. These experiments are carried out at reactor site and hence good shielding for both the sample and detector from neutron and γ-rays is required. The prompt γ-rays are assayed by high-resolution gamma spectrometry using HPGe coupled to a PC-based multi channel analyzer (MCA). Like conventional NAA method,
elemental analysis can be done by both relative and k₀-standardisation methods. In the case of relative method in PGNAA, sample and standard are irradiated separately, which is one of the major disadvantages of this technique. In the relative method analytical bias from the effects of neutron absorption and scattering in the samples and standards is experienced. Moreover one has to prepare a standard matching the sample in geometry, composition and homogeneity for every sample to be determined and carry out the experiments under identical conditions. The above problems may be eliminated by the application of k₀ standardization method in PGAA. Like in the conventional k₀-approach in NAA, sample is co-irradiated with the suitable comparator element (like Cl and Ti).

1.1.9.10 Sensitivity and Detection limits in NAA

The sensitivity (S) of an element in NAA is defined as counts µg⁻¹ for a particular condition of irradiation (ti), decay (td) and counting duration (tc). From eq.1.1, it is clear that the important parameter for better sensitivity is peak area which depends on σ, φ and ε. Since σ is fixed for an isotope, high neutron flux and higher efficiency detectors will improve sensitivity.

The smallest amount that can be determined by the activation analysis is governed by minimum activity, which can be measured reliably with sufficient precision. The minimum detectable activity of an analyte is known as its detection limit. Detection limit in NAA varies from picogram to milligram depending on the nuclear properties of the isotope of the element of interest, gamma ray background, sample matrix and pre or post chemical separations. The detection limit (L₀) although is defined as three times of the standard deviation of the background counts (C₀) under the photopeak, Currie’s expression (equation 1.7) is routinely used.

\[ L_D (counts) = 2.71 + 3.29 \times \sqrt{C_b} \]  

The counts are then converted to counts per g by using sample mass (g). Typical detection limits for many elements are computed with a flux of \(10^{13} \text{ n cm}^{-2} \text{ s}^{-1}\) and are given in IAEA TECDOC - 564 (Table 1.4).
Table 1.4: Elements and their detection limits by neutron activation analysis

<table>
<thead>
<tr>
<th>Elements</th>
<th>Detection limit/g#</th>
</tr>
</thead>
<tbody>
<tr>
<td>In, Eu, Dy</td>
<td>10^{-13} to 10^{-12}</td>
</tr>
<tr>
<td>Mn, Ru</td>
<td>10^{-12} to 10^{-11}</td>
</tr>
<tr>
<td>Co, Br, I, Sm, Ho, Hf, Re, Ir, Au, Th, U</td>
<td>10^{-11} to 10^{-10}</td>
</tr>
<tr>
<td>Na, Cl, Cu, Ga, Ge, Se, As, Pd, Sb, Te, Yb, Ta, W, Pt</td>
<td>10^{-10} to 10^{-9}</td>
</tr>
<tr>
<td>K, Sc, Ni, Rb, Sr, Y, Nb, Ru, Cd, Sn, Gd, Tb, Tm, Os, Hg</td>
<td>10^{-8} to 10^{-9}</td>
</tr>
</tbody>
</table>

# The detection limits are measured at neutron flux of $10^{13}$ n cm$^{-2}$ s$^{-1}$ and relative efficiency of 40%

1.1.9.11 Interferences in NAA

In some cases, NAA suffers from interferences, which are discussed in the following.

a) Primary and secondary interference reactions

Neutrons induce (n, p) and (n, α) reactions, in addition to (n, γ) reaction e.g. in the isotopes of low Z elements. It is possible that the radioisotopic product of the interest could be formed by more than one reaction. For example $^{28}$Al is produced by $^{27}$Al (n, γ) $^{28}$Al reaction as well as by $^{28}$Si (n, p) $^{28}$Al and $^{31}$P (n, α) $^{28}$Al reactions. If the sample matrix contains Si and P, then Al is overestimated, as the activity of $^{28}$Al is due to above three reactions. Similarly is the case of Cr estimation, if Fe is present in the matrix, it interferes the Cr estimation. Formation of products by (n, p) and (n, α) could be minimized by choosing low energy neutron spectrum of the irradiation facility as these primary interfering reactions have high energy thresholds.

Secondary interfering reactions are those, in which product formed subsequently undergo β decay and interferes in the estimations.

For example $^{30}$Si (n,γ) $^{31}$Si $\rightarrow^{31}$P (n,γ) $^{32}$P would interfere in the estimation of phosphorus.
b) Gamma-ray spectral interference

It is possible that the energies of γ-rays emitted by different activation products may be close by and interfere in the γ-ray spectrum. For example: $^{56}$Mn (activation product of Mn, $t_{1/2} = 2.58$ h) emits 846.8 keV gamma-ray and $^{27}$Mg (activation product of Mg, $t_{1/2} = 9.45$ min) emits a gamma-ray of 843.8 keV. Although there is considerable improvement in the resolution of HPGe detectors, with capabilities of resolving these gamma rays, still these two γ-rays overlap. Since the difference is substantial, contribution from both the nuclides could be resolved by spectrum deconvolution using programs like SAMPO. By taking advantage of difference in the half-lives of the radionuclides, indpendant contributions from $^{56}$Mn and $^{27}$Mg could be obtained. The lower half-life nuclide is measured using short irradiations and shorter cooling and counting periods. With longer cooling periods, activity due to long lived isotopes can be measured. If the half lives are comparable and the γ-energies are unresolvable, radiochemical separations have to be resorted to.

c) Self shielding of neutrons by the sample

If the samples contain elements with very high neutron capture cross-section (Cd, B etc.) then the loss of neutrons during the irradiation is considerable. This is known as neutron selfshielding. Thus the net neutron flux is less than the expected flux in the irradiation position, which may lead to the underestimation of the analytes. Self shilding can be minimized by keeping sample size as small as possible, so that the flux remains nearly constant throughout the sample.

d) Absorption of γ-rays in the sample and other uncertainties during counting.

In the case, where samples are bulky and contain high Z elements, it is possible that γ-rays are attenuated in the sample and this effect is known as self shadowing. This effect is more pronounced in the case of low energy γ-rays. Self shadowing could be minimized by keeping the size of the sample and standard as small as possible. To avoid possible coincidence effects, irradiated samples are measured by keeping them at 12-15 cm from the detector.
1.1.9.12 Advantages of NAA

1. The technique has high sensitivity for many elements in the periodic table.

2. NAA is based on nuclear properties of the analyte and hence the technique is independent of the chemical state of the analyte.

3. NAA is a simultaneous multielement analysis technique.

4. It is generally a non-destructive technique, unless there is a special need.

5. In many cases, the technique is capable of rapid analysis, particularly when short-lived radionuclides are of interest.

6. A unique and most important advantage of NAA technique is its freedom from contamination. In the case of INAA, the sample is not subjected to chemical treatment prior to the irradiations and in the case of RNAA, elemental contamination in post irradiation radiochemical treatment does not affect the measurement of induced radioactivity. The technique is also free from sample/process blanks.

1.1.9.13 Limitations of NAA

Some of the drawbacks that NAA suffers are given below-

1. This methodology needs a neutron irradiation facility with high flux such as a nuclear reactor and it is limited to a very few research organizations.

2. Very short-lived radionuclides, with half-lives in the range of a few seconds, formed in neutron irradiation are difficult to detect. There is always a time lag between the end of irradiation and start of counting. But with the advances in the technology such as pneumatic carrier facility (PCF), it is now possible to measure isotopes with half-lives of few tens of seconds.

3. For some elements like phosphorus, the product is a pure $\beta^-$ emitter and $\beta^-$ counting is non specific as the sample contains many elements and the products formed are $\beta^-$ unstable.

4. Long irradiations followed by long counting periods are required for isotopes with long half-life. In such cases, sufficient cooling time has to be given. This process is very time consuming and may take a few weeks to complete.
5. Information about the chemical species present in the sample can not be obtained in NAA.

6. Conventional NAA is not suitable for certain elements like Si, P, Pb, B, Gd and low Z elements.

1.2 Bioavailability of trace elements from plant food.

Plant based foods are major sources of essential trace elements in all parts of the world. Mineral content in edible portion of the plants is influenced by many factors such as plant genotype, soil properties, environmental conditions and nutrient interaction. Within agricultural scientific community it is widely accepted that the total micronutrient content of the soil does not indicate the amount of micronutrient ‘available’ to the plant for growth and other necessary physiological actions. Similarly all minerals present in plant food are not bioavailable for absorption and/or utilization for normal health and normal physiological functions. ‘Bioavailability’ is defined as the fraction of total content available for absorption by humans. Chemical and biochemical, in vitro and in vivo methods have been developed to determine the bioavailability.

1.2.1 Definitions for bioavailability:

Bioavailability is not the fundamental property associated with the plant food but represents the response of the test subjects (humans, animals, cell cultures etc.) to the diet. Different researchers working in this area defined the term bioavailability in different ways. ‘The proportion of total mineral content in the food utilized for normal body functions and growth’ is known as bioavailability. Bioavailability reflects the efficiency with which consumed nutrients are absorbed from alimentary tract and are thus available for further use. Ammerman et al. defined bioavailability as the ‘degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal’. Similarly bioavailability refers to that portion of total amount of mineral present in the food that is potentially absorbable in a metabolically active form. The ‘metabolically active form’ or ‘potentially absorbable amount’ of micronutrients or the biological response (i.e. bioavailability) may be different for different animals and several factors such as food composition, food preparation or
processing and host factors. The factors listed below may affect bioavailability\textsuperscript{63} of minerals.

1. Protein quality (protein source, animal and plant protein, amino acids etc)
2. Quantity of trace element (Total amount)
3. Physico-chemical form (Oxidation state)
4. Nutrient interaction (element-element, element-organic component)
5. Presence of promoters in food (ascorbate, citrate, vitamins, amino acids, sugars etc.)
6. Inhibitors (Oxalates, phytates, polyphenols, fiber etc.)

Food preparation, processing and form in which they are consumed also influence bioavailability.

In addition to this status of the host e.g. nutritional status (malnutrition, overnutrition, deficiency etc.), physiological status (pregnancy, lactation etc.), economic status (type, quality and quantity of food selected for diet) also influence bioavailability.

1.2.2 Methods for determination of bioavailability of elements

1.2.2.1 Radioisotopic methods//In vivo methods

Since late 40's\textsuperscript{64, 65}, methods to assess trace element bioavailability are being developed. Both in vivo and in vitro techniques have been used to obtain mineral bioavailability. But no one procedure is adequate to determine bioavailability of all elements. In vivo techniques generally utilize the radiotracers. Most of the studies were carried out on Fe and Zn\textsuperscript{66}. These procedures generally measure retention of either stable or radioactive isotope provided in test meals to the test subject. Then bioavailability is calculated as a difference between the intake (ingestion) and excretion. In these retention studies, test meals are labeled with tracer added either intrinsically or extrinsically to entire meal or to the specific component in the meal. Extrinsic labeling consists of adding the isotope, usually in the form of an inorganic isotope, directly to the food that is to be evaluated. Intrinsic labeling involves the biological incorporation of the isotope in the edible plant part during the growth of the plant. The advantage of this technique is that,
the isotope is biologically incorporated in the plant in the same manner and associated with the same constituent as it occurs naturally.

1.2.2.2 In vitro methods

If bioavailability studies are carrying out by in vitro simulation methods, then the term bioaccessibility is used. There is a considerable demand for developing rapid and inexpensive in vitro procedures to estimate the bioavailability. Most of the in vitro procedures involve simulating the gastric and gastro-intestinal digestive conditions in the laboratory and extracting the minerals in those digests and estimating them later on. Bioavailability is calculated as difference in the total and that present in the digest. In vitro methods cannot precisely predict, how much of a nutrient will be absorbed and utilized by the human subjects. However these techniques can serve as preliminary tools to identify the most potential species, processing techniques and growing conditions to help increasing bioavailability.

The first report on bioavailability of iron by in vitro digestion was reported by Rao and Prabhawati. They developed a procedure in which food or meal was incubated with pepsin-HCl gastric digestive juice at pH = 1.75 and temperature 37°C. The digestion was followed by centrifugation and filtration. Aliquot of the supernatant of the digest was adjusted to pH=7.5 and ionizable iron in the filtrate was determined spectrophotometrically by extraction with α-α dipyridyl. Supernatants obtained after centrifugation in this procedure are not clear liquids, causing problems in the estimation of minerals.

Gastro-intestinal digestion at pH = 7.0 is the next step in the in vitro method. The most important step in this procedure is to adjust the gastric pH =1.75 to gastro-intestinal pH (7.0). It is found that the pH adjustment is one of the major sources of variability in estimating the bioavailability of minerals by this method. This problem was offset by placing an appropriate amount of NaHCO3 in a dialysis bag (with protein cut off 10000-12000 kDa) and allowed to diffuse in the gastric digest. Iron and zinc are the most studied, however scarce information is available on bioavailability of other trace elements such as Mn, Cu, I, Ca and Mg.
1.3 Reactive oxygen species and Antioxidant Activity

1.3.1 Free Radicals

Free radical is ‘any chemical species capable of independent existence that contains one or more unpaired electrons’\(^7\). They are paramagnetic. They are highly reactive and reducing species and the chemical reactivity varies from species to species. The free radicals can be formed by loss of an electron from non-radical.

\[ X \rightarrow e^- + X'^* \]

Radical cation

By gaining an electron, a non-radical species becomes a free radical.

\[ X^- + e^- \rightarrow X'^- \]

Radical anion

Radical can also be formed when a covalent bond is broken and two electrons are shared by both of the atoms associated with the bond. This process is called as hemolytic fission. Covalent bonds can be broken only at high temperatures in the range of 177 to 325 °C.

\[ X : Y \rightarrow X'^* + Y'^* \]

For example, hydrogen and hydroxide free radicals are formed by homolytic fission of water.

\[ H_2O \rightarrow H'^* + OH'^* \]

1.3.1.1 Reactive oxygen species

The presence of free radicals in biological systems was realized in 1954\(^8\). Most of the free radicals generated in the body are oxygen derived and hence the name reactive oxygen species (ROS). This is a collective term used to denote oxygen free radicals (O\(_2^*\) and OH\(^*\)) as well as some non-radical species such as H\(_2\)O\(_2\) and hypochlorous acid (HOCl)\(^9\). Sometimes reactive oxygen species are also called as oxidants. Reactive oxygen species that are produced \textit{in vivo} are given in Table 1.5.
Table 1.5: Reactive oxygen species

<table>
<thead>
<tr>
<th>Free radicals</th>
<th>Non-radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>O$_2^-$</td>
</tr>
<tr>
<td>Hydroxyl</td>
<td>OH$^-$</td>
</tr>
<tr>
<td>Alkyl peroxyl</td>
<td>RO$_2^*$</td>
</tr>
<tr>
<td>Alkoxyl</td>
<td>RO$^*$</td>
</tr>
<tr>
<td>Hydroperoxyl</td>
<td>HO$_2^-$</td>
</tr>
</tbody>
</table>

1.3.1.2 Reactive nitrogen species

In biological systems reactive nitrogen species (RNS) are equally significant as ROS. Reactive nitrogen species such as nitrosonium cation (NO$^+$), nitronyl anion (NO$^-$), or peroxynitrite (ONOO$^-$) are some of the reactive nitrogen species (RNS).

1.3.2 Generation of ROS in vivo

If a single electron is added to ground state O$_2$ molecule, it enters one of the antibonding $\pi^*$ orbitals forming superoxide radical (O$_2^-$). Of all the ROS, superoxide is the most toxic. Following are the components and processes by which O$_2^-$ is generated in the body.

i) Mitochondrial electron transport

ii) Enzymes

iii) Auto-oxidation reactions

iv) Heme proteins

v) Mitochondrial DNA

Out of these processes, mitochondrial electron transport is the most important source of O$_2^-$ in vivo. Generation of mitochondrial ROS is a consequence of oxidative phosphorylation which results in the synthesis of ATP (Adenosine triphosphate) molecules (Fig. 1.7). Mitochondria synthesize ATP by oxidation of food through the respiratory chain. During metabolism, the complete oxidation of glucose and fatty acids (the principal sources of energy in animals and for most of the non-photosynthetic cells) to CO$_2$ and water results in synthesis of 32 molecules of ATP. In addition, reduction of
oxygen to water takes place by consecutive transfer of four single electrons. During each step of electron transfer, three reactive oxygen species namely superoxide (O₂⁻), hydroxyl (OH⁻) and hydrogen peroxide (H₂O₂) are generated⁸⁰⁻⁸³ (Fig. 1.8).

Fig. 1.7 Generation of ROS in mitochondrial electron transport and enzymatic antioxidant reactions⁷⁹

Fig. 1.8 Generation of ROS during the reduction of oxygen to water during photosynthesis⁸¹⁻⁸³
1.3.3 Oxidative Stress and Free radicals

In healthy living organisms, there is enough endogenous antioxidant defense mechanism to cope up with normal physiological rate of ROS/RNS generation. This balance of generation of ROS/RNS and the activities of endogenous antioxidant defense system is delicately maintained in healthy organisms. Oxidative stress is a situation that refers to the serious imbalance between production of pro-oxidants and the potential of antioxidants, shifting in favor of the former. This shift can be due to physiological states such as pregnancy\(^84\), \(^85\) viral infection and external factors such as environmental pollution, cigarette smoke, automobile exhaust, radiations and air pollution\(^86\). In addition to this, the oxidative stress can also result from i) diminished endogenous antioxidant defense enzymes (e.g. CuZnSOD, MnSOD and glutathione peroxidase) or the diseases that deplete such defense system and ii) increased production of ROS/RNS (e.g. by exposure to elevated O\(_2\), the presence of toxins that are themselves reactive species (NO\(_2^+\)) or these toxins are metabolized to generate ROS/RNS.

Free radicals induced oxidative stress can damage cells through various mechanisms like direct oxidative damage to DNA, lipids and proteins, depletion of adenosine tri phosphate (ATP) and Nicotinamide adenine dinucleotide (NAD\(^+\)), activation of poly (ADP-ribose) synthetase (PARP), fall in GSH (glutathione peroxidase reduced)/GSSG (Glutathione peroxidase oxidized) ratios. Fig. 1.9 depicts the oxidation stress to the different cell components like cell nucleus due to excessive generation of ROS inside the cell. The oxidative stress induced by excessive generation of free radicals can result in the diseases such as neurodegenerative (Parkinson’s and Alzheimer’s), arteriosclerosis, hypertension, diabetes mellitus and hyperglycemia, ischaemia-reperfusion, chronic inflammatory diseases, rheumatoid arthritis, inflammatory bowel disease, various types of cancers, tumours as well as the process of ageing.
1.3.4 Antioxidant Defence System

Antioxidants are substances, which can prevent the activity of the free radicals generated in the body. Antioxidant defence system comprises defence from two groups of antioxidants. One is from endogenous antioxidant enzymes such as superoxide dismutases (SODs), catalase and glutathione peroxidase along with some metalloproteins such as albumin, ceruloplasmin and ferretin. The SODs are primarily of three types and they are Mn-SOD, Cu-ZnSOD and Fe-SOD. The other group comprises of low molecular compounds such as carotenoids, flavonoids, polyphenols and many vitamins from the diet.

1.3.4.1 Endogenous Antioxidant Enzymes
1.3.4.1.1 Superoxide Dismutase (SOD)

The presence of erythrocyte protein is realised in 1969, which has sole function of removing the superoxide radical catalytically and converting it to hydrogen peroxide. These proteins are termed as superoxide dismutase. Cu-ZnSODs are present almost all eukaryotic cells along with Mn-SOD. The mechanism by which they dismute the superoxide is shown below.
Enzyme – M^{n+} + O_2^* → Enzyme – M^{(n-1)+} + O_2

Enzyme – M^{(n-1)+} + O_2^* + 2H^+ → Enzyme – M^{n+} + H_2O_2

Net Reaction: O_2^* + O_2^* + 2H^+ → H_2O_2 + O_2

Where M^{n+} represents the metal ion in the enzyme, like Cu in Cu-ZnSOD and Mn in Mn-SOD.

1.3.4.1.2 Catalase

The end product of superoxide dismutase is hydrogen peroxide. It is removed by two types of enzymes in aerobes catalase and glutathione peroxidase. The catalase contains Fe(III) which helps hydrogen peroxide to molecular oxygen shown below-

Catalase – Fe(III) + H_2O_2 → Compound – I + H_2O + \frac{1}{2}O_2

Compound – I + H_2O_2 → Catalase – Fe(III) + H_2O + \frac{1}{2}O_2

Net Reaction: 2H_2O_2 → 2H_2O + O_2

1.3.4.1.3 Glutathione Peroxidase

Glutathione peroxidase (GSH) present in animal tissue removes H_2O_2 by reducing it to H_2O and in the process it gets oxidized. The reaction can be written as

H_2O_2 + 2GSH → GSSG + 2H_2O

1.3.4.2 Antioxidants from Diet

Epidemiological studies indicated that taking a diet that is rich in fruits and vegetables decreased the risk of cardiovascular diseases and certain forms of cancer. Phytochemicals, especially polyphenolics and bioflavonoids, present in the fruits and vegetables are identified as major bioactive components responsible for health benefits. These phytochemicals scavenge free radicals and oxidants and hence can be used to estimate the antioxidant activities of food products, medicinal plants, etc. For this
purpose protocols have been developed and some of them are discussed in the following sections.

1.3.5 Chemistry and mechanism involved in assays used for dietary antioxidant activity

There are two mechanisms to deactivate free radicals generated in vivo. They are Hydrogen atom transfer (HAT) and single electron transfer (SET). In HAT mechanism, the antioxidant action takes place via transfer of hydrogen atom from antioxidant to the free radical. Hydrogen can easily be transferred to free radicals if bond dissociation energy (BDE) is lower. In the case of SET mechanism, antioxidant action takes place via transfer of single electron from antioxidant to the free radical. In this mechanism, ionization potential (IP) of the antioxidant molecule is most important.

1.3.5.1 HAT-based methods

In the methods based on HAT, ability of an antioxidant (AH) to quench free radicals through hydrogen donation is measured. Any hydrogen donor molecule acts as an antioxidant. However, a good candidate is the one, when used, does not lead to side reactions by the free radicals formed at the end of reaction. Following is a general reaction for HAT mechanism. In the following reaction $X^*$ is a free radical that reacts with an antioxidant molecule AH.

$$X^* + AH \rightarrow XH + A^*$$

The BDEs of the most of the known antioxidant are generally in the range of 8–10 kcal/mol. The reactions based on HAT mechanism are fast (complete within a few seconds) but the presence of extra reducing agents, including metals can lead to underestimation of BDE and thus antioxidant activity. Oxygen radical absorbance capacity (ORAC) is an important method to check the antioxidant activity of test sample that follows the HAT mechanism.
1.3.5.2 SET-based methods

In the methods based on SET, the ability of an antioxidant to quench free radical through electron donation is measured. Following set of reactions give the mechanism by which free radical is quenched through electron donation by antioxidant molecule, AH.

\[ X^* + AH \rightarrow X^- + AH^{++} \]
\[ AH^{++} + H_2O \leftrightarrow A^- + H_3O^+ \]
\[ X^- + H_3O^+ \rightarrow XH + H_2O \]

The antioxidant activity depends on deprotonation capacity and ionization potential (IP) of a particular functional group in the antioxidant, and hence SET based reactions are pH dependant. The antioxidant assays such as Ferric reducing antioxidant power (FRAP) and Folin-ciocalteu method (total phenolic content assay) are routinely used for measuring the antioxidant power.

In 1,1'-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and inhibition of generation of 2'-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical assay, stable free radicals are used to measure the antioxidant activity. In such cases, both HAT and SET mechanisms are operative.

1.3.6 Total Antioxidant Activity

1.3.6.1 Spectrophotometric Assays

1.3.6.1.1 Ferrylmyoglobin/ABTS'⁺ Assay

Miller and Rice-Evan originally developed this assay. It is based on the radical scavenging capacity of the antioxidant extract. The ABTS'⁺ is produced by reacting ABTS diammonium salt with suitable oxidant. This anion radical has intense absorption peaks at 415, 645, 734, and 815 nm. The measurements are generally carried out at 415 and/or 734 nm. In this metmyoglobin and H₂O₂ produce ferrylmyoglobin, which, reacts with ABTS salt to generate ABTS’⁺ radical cation. The mechanism by which ABTS’⁺ radical cation is generated and reacts with antioxidant extract is shown in the Fig 1.10. Antioxidant extract is added to reaction mixture prior to generation of radicals and check the efficiency of the extract to delay the radical generation time. In the post addition method, free radicals are initially generated and the absorbance of the radical at the
respective wavelengths\textsuperscript{93, 94} is measured. In both the cases trolox is used as standard antioxidant and antioxidant capacity can be expressed as trolox equivalent antioxidant capacity (TEAC). Pre-addition method suffers from a drawback that, antioxidant extract may react with the oxidant added to generate ABTS\textsuperscript{**} radical (ferrylmyoglobin) leading to the overestimation of antioxidant potential of the sample. In some cases post addition methods are preferred. However, in this case, it is difficult to detect the completion of the reaction as the extract is a mixture of many antioxidants and the reaction kinetics for different antioxidants may be different. Advantages and disadvantages of the method are listed below

Advantages-
1. The methodology is very simple and the results are reproducible.
2. The reaction time of ABTS\textsuperscript{**} with the antioxidant is less (typically 30 min.), ABTS\textsuperscript{**} is stable over a wide range of pH\textsuperscript{95}.
3. ABTS is soluble in water as well as in alcohol and thus can be used for aqueous as well as organic extracts.

Disadvantages-
1. ABTS\textsuperscript{**} radical cation produced is not found in organisms hence sometimes it is ‘nonphysiological’.
2. The redox potential of ABTS is 0.68 V hence thermodynamically any compound whose redox potential is less than this value can reduce ABTS\textsuperscript{**}.

Fig 1.10 Antioxidant mechanism in Ferrylmyoglobin/ABTS assay
1.3.6.1.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay gives the capability of the sample to reduce Fe (III) to Fe(II). The assay was originally designed by Banzie and Strain\textsuperscript{96,97} to measure the reducing power of the plasma, but subsequently the assay was utilized to check the antioxidant power of medicinal extracts and botanical samples. In the reaction, TPTZ is reacted with Fe (III) to form a Fe (III)-TPTZ complex\textsuperscript{91} as shown in the Fig 1.11. The complex does not absorb visible or UV light. When a reducing antioxidant is added to the complex solution, Fe (III) gets reduced to Fe (II) forming Fe (II)-TPTZ complex by electron transfer. This complex is intense colored with absorption maximum at 595 nm. From the color intensity, the reducing power of the antioxidant extract is calculated by using a calibration curve obtained from standard antioxidants such as Trolox and ascorbic acid. Further the results are expressed as TEAC and AEAC respectively.

\begin{equation}
\text{FRAP} = \frac{\text{Absorbance at } 595\text{ nm}}{\text{Concentration of antioxidant}}
\end{equation}

Disadvantages and advantages- It is often argued that, FRAP measures only the reducing ability of the antioxidant extract to reduce Fe(III) and not relevant to antioxidant activity definition as it has no direct relationship with radical quenching phenomenon; mechanistically and physiologically. But oxidation or reduction of radicals to ions can still be considered as an important step in radical chain breaking mechanism. The reaction is too fast and reaches the end point in 4-6 min\textsuperscript{98}. Fast reacting phenols that rapidly bind the iron can be best studied by this method. When the extracts contain phenols that react slowly with the Fe (III)-TPTZ complex, they do not contribute to the overall FRAP value. FRAP method is simple, rapid, inexpensive and does not require sophisticated instrumentation.
1.3.6.1.3 DPPH free radical scavenging assay

DPPH is one of the stable free radicals and commercially available. The unpaired electron is present on the nitrogen atom (Fig 1.12). It is easily soluble in ethanol or methanol. It is purple in color and absorbs at 515 nm. Since it is a free radical, its reaction can also be followed by electron spin resonance (ESR) spectrometry. The assay requires just appropriate concentration of DPPH solution and test antioxidant extract. When an antioxidant extract is added to the DPPH solution, its concentration decreases either by accepting electron or hydrogen atom from antioxidant and results in the decrease of the absorbance. The reaction is shown in Fig. 1.12. The amount of DPPH remained after completion of the reaction, (the period is generally 30 min.) is calculated. A calibration curve is plotted using standard antioxidants such as trolox or ascorbic acid from which, the antioxidant potential of the test extract is calculated.

Advantages and disadvantages: This is a simple and rapid method and is one of the most widely used assays to screen the extracts for their antioxidant potency. The antioxidants present in the extract may also have absorbance in the region where DPPH absorbs. This is the case with carotenoids, because of which DPPH antioxidant values may be overestimated. DPPH is stable nitrogen centered free radical and does not have any similarity with actual free radicals generated in vivo.

![Fig 1.12 Antioxidant mechanism of DPPH free radical scavenging assay](image)

1.3.6.1.4 Folin-Ciocalteu (F-C) or Total Phenolics and flavonoid Assay

This method was originally developed to quantify proteins. Folin-Ciocalteu reagent is reactive to tyrosine, which has phenolic residue. The mechanism is based on
oxidation-reduction and therefore it can be used for antioxidant assay. Singleton et.al.\textsuperscript{102} optimized and extended the method to quantify total phenolic content in wines. Since then F-C reagent method is commonly used to detect and quantify the total phenolic content of the natural products.

The F-C reagent is prepared by boiling the mixture of sodium tungstate (Na\textsubscript{2}W\textsubscript{O}\textsubscript{4}.2H\textsubscript{2}O, 100g), sodium molybdate (Na\textsubscript{2}Mo\textsubscript{O}\textsubscript{4}.2H\textsubscript{2}O, 25g), concentrated hydrochloric acid (100 mL), 85\% perchloric acid (50 mL) and water (700 mL) for 10 h. After boiling, 150g of lithium sulphate (Li\textsubscript{2}SO\textsubscript{4}.4H\textsubscript{2}O) is added until yellow colour is formed. The exact nature of F-C reagent is unknown but believed to contain heteropolyphosphotungstates-molybdates. Its reduction leads to a blue colored species, possibly [PMo\textsubscript{W}\textsubscript{11}O\textsubscript{40}]\textsuperscript{4-} as a result of electron transfer as shown below.

\[
\text{Mo\ (VI) + e}^{-} \rightarrow \text{Mo\ (V)}
\]

Yellow \hspace{1cm} Blue

Major criticism to this method is that, any component capable of donating electron to F-C reagent will give rise to blue colour leading to an overestimation of the actual phenolic content. In order to overcome this limitation, Singleton et.al.\textsuperscript{102} modified the method by addition of alkali (Na\textsubscript{2}CO\textsubscript{3}) for specific binding to polyphenols. Following steps are to be ensured to estimate the total phenolic content in antioxidant extracts: 1) Proper ratio of test alkali to F-C reagent, 2) optimal reaction time and temperature for color development, 3) monitoring the optical density at 765 nm and 4) use of gallic acid as a reference standard phenol.

1.3.6.1.5 Lipid peroxidation

Lipid peroxidation is an important antioxidant assay as it is close to human systems\textsuperscript{103, 104}. In this test, rat mitochondria is used. In lipid peroxidation \textit{in vivo} can be initiated by any harmful substance or metal atoms such as iron and copper. It consists of three steps namely initiation, propagation and termination under antioxidant conditions. The schematic representation of lipid peroxidation is shown below\textsuperscript{105}.  

\[
\begin{align*}
A + LH + O_2 & \rightarrow LO_2 \\
LO_2^* + LH & \rightarrow L^* + LOOH \\
L^* + O_2 & \rightarrow LO_2^* \\
LO_2^* + LO_2^* & \rightarrow \text{products} \\
LOOH^* & \rightarrow \text{F.R.} \\
QH_2 + LO_2^* & \rightarrow QH^* + LOOH \\
QH^* + LO_2^* & \rightarrow \text{products}
\end{align*}
\]

where A is the substance that initiates the oxidation of lipid LH. In steps 2, 3, 4 and 5 oxidation of lipid triggered by A is propagated. When phenolic antioxidant such as QH$_2$ is added, the chain of formation of free radicals is inhibited.

1.3.6.2 Spectroflorometric assay

1.3.6.2.1 ORAC assay

Ghiselli et.al\textsuperscript{108}, Glazar et.al\textsuperscript{109} and Cao et.al\textsuperscript{110} developed the oxygen radical absorbance capacity (ORAC) assay. ORAC is a measure of the inhibition of peroxide radical induced oxidative damage. Thus ORAC is an example for radical chain of breaking antioxidant activity by hydrogen atom transfer (HAT) mechanism\textsuperscript{111}. In this assay, peroxide radical reacts with fluorescent probe, which on oxidation forms non-fluorescent product. By measuring the decrease in the fluorescence and comparing it with reference standard antioxidant (Trolox) can quantify antioxidant activity. The antioxidant action mechanism\textsuperscript{91} can be shown as follows-

\[
\begin{align*}
R-N=N-R + O_2 & \rightarrow N_2 + 2\text{ROO}^* \\
\text{ROO}^* + \text{Probe (Fluorescent)} & \rightarrow \text{ROOH} + \text{Oxidized Probe (No fluorescence)} \\
\text{ROO}^* + AH & \rightarrow \text{ROOH} + A^* \\
\text{ROO}^* + A^* & \text{Fast} \rightarrow \text{ROOA}
\end{align*}
\]
A protein, β-phycoerythrene, is used as a fluorescent probe and 2.2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) is used as a peroxide radical generator. The protein gets oxidized and florescence decreases. As kinetics is reasonably fast, it is a convention that the area under the time decaying absorbance profile is taken as the direct signal for antioxidant activity. To ensure this, reaction is followed (decrease in florescence) for more than 30 min.

1.4 Literature Survey on wheatgrass

It is known since long that nutritional value of sprout is higher than that of cereals and legume grains. This is particularly true in the case of wheat (*Triticum aestivum* L.). Sprouting of wheat seeds for a limited period causes an increase in activities of hydrolytic enzymes, improvement in the contents of certain essential amino acids, total sugars, B vitamins, and decrease in dry matter, starch, and antinutrients such as phytic acids. The magnitude of the nutritional improvement is, however, influenced by the type of cereal, seed quality and sprouting conditions. Kadam and Chavan[112] critically reviewed the literature on improvements in the nutritional qualities of cereal sprouts including wheatgrass/wheat sprouts.

Lai et al.[113] were among the first to report the potential of wheatgrass from medicinal point of view. They found that extracts from the roots and the leaves of wheat sprouts selectively inhibited the mutagenic effect of carcinogens requiring metabolic activation as demonstrated in the Ames Salmonella/mammalian microsome test. Formation of dihydrodiol metabolites of benzo[a]pyrene was found to be decreased. Later Lai and Anderson independently found that chlorophyll is the active factor in wheat sprout extract inhibiting the metabolic activation of carcinogens *in vitro*. The fraction containing high amounts of powerful antioxidant glycoside molecule has been isolated by thin layer chromatography. A protective effect of this wheat sprout fraction on the oxidative damage of pBR322 plasmid DNA was also demonstrated. The results in this report showed that, the concentrations of antioxidant compounds strongly increase during the germination phase[114].

It is shown recently by Calzuola et al.[115] and Marseli et al.[116], that wheatgrass (Wheat sprouts of 5-6 days old) synthesizes antioxidant compounds during germination.
The authors analyzed, the aqueous and ethanolic extracts obtained from wheat sprout powders for their antioxidant activities. In addition to this, it was shown that, the oxygen superoxide scavenging activity performed by 1 g sprout powder was comparable to that shown by 10 mg pure antioxidant compounds such as rutin and quercetin. Biochemical analysis of the sprout extracts showed that the antioxidant activity is mainly due to the presence of reducing glycosides and polyphenolic compounds present in sprouts synthesized during germination.

Yang et al studied the antioxidant content of wheat grains to determine the optimum period that would maximize the production of antioxidants in wheat sprouts. The changes in concentration of antioxidant compounds such as vitamins C and E, β-carotene, ferulic acid and vanillic acid were monitored over the germination period. These antioxidant compounds are absent or barely present in dry wheat seeds. However, upon germination, the concentration of these compounds increase steadily with increasing germination period reaching their peaks after 7 d. They concluded that, wheat grains germinated for 7 d would produce the wheatgrass with optimum concentrations of antioxidant.

Aqueous extracts of wheatgrass of 11-12 d were studied for their antimutagenic effect induced by benzo[a]pyrene in strain TA98 of Salmonella typhimurium in Ames test. It was found that the extracts of wheatgrass contain heat resistant compounds showing remarkable antimutagenic activity. In addition to this, same researchers found in their animal studies that sperm abnormalities induced by benzo[a]pyrene in mice were diminished after oral administration of wheatgrass extracts.

Very less literature is available on the elemental content of germinating wheat (Wheatgrass). Lintschinger et al studied the uptake of various trace elements during germination of wheat, buckwheat and quinoa. As a control for possible changes in the biochemical metabolism of the sprouts, the biosynthesis of vitamin C was also determined by using reversed-phase ion-pair HPLC. It was shown that quinoa was the most resistant to the applied electrolyte solutions and had the highest uptake rates for almost all elements, followed by buckwheat and wheat. Maximum increase was observed for Co, Sr, and Li. No significant change in vitamin C biosynthesis was observed between sprouts grown in different electrolyte solutions. The time-dependent uptake for
most elements was characterized by a significant absorption during soaking of the seeds, followed by a lag phase during the first day of germination and an increased uptake during the second and third days. Se and As showed distinctly different uptake behaviors.

Lintschinger et al. studied the effect of process of sprouting as an alternative to enrich selenium in wheat. Sprouting was chosen because it improves the nutritional value of seeds by higher vitamin content, by better quality of protein, and some other parameters. Wheat, alfalfa (*Medicago sativa*), and sunflower (*Helianthus annuus*) seeds were germinated for 5 and 7 days in solutions containing selenate. The selenium sensitivity of the sprouts was tested by measuring visible germination levels and seedling development. Uptake rates were studied by determination of total selenium using inductively coupled plasma mass spectrometry (ICP-MS). Metabolism of the absorbed selenium was analyzed by determination of selenium species in extracts of the sprouts using anion exchange HPLC coupled to ICP-MS. Wheat and alfalfa were less resistant and enriched selenium up to concentrations of 100 and 150 mg of Se/kg of dry mass, respectively. With this method it was concluded that, it is possible to produce sprouts containing higher amounts of selenium, which might provide substantial proportions of selenium in a bioavailable form.

A few clinical trials have been performed on wheatgrass juice to realize its potential in prevention and cure of various diseases. In a randomized, double blind and placebo-controlled study in Israel, on the patients suffering from distal ulcerative colitis, the treatment with oral administration of wheatgrass juice (100 mL of fresh wheatgrass juice for 1 month) was associated with significant reduction in the overall disease activity index and in the severity of rectal bleeding. The authors observed no serious side effects. Further in an attempt to demonstrate the compounds responsible for observed effect by cyclic voltametry, they observed four prominent peaks corresponding to four groups of compounds that exhibit antioxidative properties in fresh wheatgrass juice. These peaks found to decrease in number and amplitude over time.

Marwaha et al. demonstrated that fresh wheatgrass juice reduces transfusion requirements in patients suffering from thalassemia major. The families of patients raised the wheatgrass at homes in kitchen garden/pots. The patients consumed about 100 mL of wheatgrass juice daily. Pre-transfusion hemoglobin; amount of blood transfused and body
weight were recorded in the interval between transfusions. It was observed that, juice prepared from fresh wheatgrass is effective with respect to all the above recorded variables.

1.5 Scope of the present work

Since long time, wheatgrass is being consumed believing that it has medicinal properties. A brief literature survey mainly focuses on the medicinal properties of wheat sprouts. Potential of wheatgrass/wheat sprouts as a medicinal herb was reflected in the works of most of the authors.

No reports are available in literature on total elemental content of wheatgrass from nutritional supply point of view. In view of its health beneficial aspects it is of worth to know its mineral content. In the present study, a systematic investigations were made on mineral content, their bioaccessibility and antioxidant activity of wheatgrass. Wheatgrass growth was monitored over a period of 20 days. Elemental concentrations in wheat seeds and shoots (grass) and roots (root + remains of seeds) of wheatgrass collected periodically during 5-20 days of growth are determined. The elemental concentration levels, their trends and ratios of elements in shoot-to-root are discussed. Additionally, a set of commercially available wheatgrass tablets, used as a dietary supplement, were analyzed. Five biological reference materials obtained from various standardizing agencies such as NIST, INST and IAEA were also analyzed to evaluate the accuracy of the method.

In the present work, the bioaccessibility of some essential minerals (Ca, Mg, Mn, Zn and Na) from wheat seeds, fresh wheatgrass and commercially available wheatgrass tablet is studied by modified in vitro gastric and gastro-intestinal digestion methods.

It is fairly well understood that, most of the diseases and disorders related to the human body are due to oxidative stress. This is a physiological condition in which, delicately maintained balance between production of reactive oxygen and nitrogen (ROS and RNA) and activity of endogenous antioxidants in vivo shifts in favour of the former. Under such conditions, a diet rich in antioxidant compounds can be helpful. In view of this antioxidant activity of wheatgrass was investigated. Though there are some preliminary studies, the antioxidant activity of wheatgrass, at various levels of protection,
has not been studied in detail. It is also not known at what period of growth the wheatgrass has maximum antioxidant potential. The effect of different germination conditions used for cultivation of wheatgrass such as nutrients and soil also is not studied. In the present study, the antioxidant potential of wheatgrass, at different levels of action, during its germination period under different growth conditions has been experimentally evaluated. The possible factors responsible for the differences, in terms of the chemical composition, observed were also examined.