CHAPTER 2
Materials and Methods
Section – 1: Chemicals and their properties

2.1.0. Introduction: A brief account on the properties and significance of the chemicals used in the present research investigation is briefed in this chapter.

The present investigation involves the synthesis, characterization of water soluble low and high molecular weight polyelectrolytes, such as sodium salt of acrylic acid with acrylamide, which are generally termed as polyacrylamide or copolymer of acrylic acid and acrylamide and also polystyrene sulfonic acid. These are anionic polyelectrolytes, which are derived from polyacrylamide by controlled hydrolysis.

The molecular weight of synthesized polyelectrolytes, were determined by viscosity measurement method by using 1,4 - dioxane as solvent.

2.1.2. Materials:

2.1.2.1. Acrylamide: Acrylamide is available\(^4\) as a high molecular weight material that is soluble in water nearly under all conditions. Co-polymers can be made easily with most other monomers, thus extending the range of properties and applications. Polymer derived from the substituted monomers include clear gels, soft gums, and hard plastics. By further reaction at the amide function one can convert many of the polymers into polyelectrolyte or into thermosetting resins.

Acrylamide is the parent compound (monomer) for poly acrylamid, which is heavier molecular formula \(\text{CH}_2=\text{CHCONH}_2\), and possess a melting points of 84.5°C.

In the present work acrylamide is used for the synthesis of high molecular co-polymer of sodium salt of acrylamide.

2.1.2.2. Acrylic acid: Acrylic acid\(^4,73\) is colourless liquid with sharp penetrating odour, that resemble the odour of acetic acid. Acrylic acid soluble in water,
methanol, ethanol, benzene and other organic solvents. Its molecular formula \( \text{CH}_2=\text{CHCOOH} \). Its polymer polyacrylic acid has the molecular weight ranging from 10,000 to several million. Acrylic acid polymer or acrylic acid –acrylamide co-polymers with molecular weight above 300,000 are generally used as flocculants in sugar industries.

In the present work acrylic acid is used for the syntheses of high molecular weight co-polymer of sodium salt acrylamide.

2.1.2.3. **Styrene:** It soluble in cyclohexan, benzen, toluene, eithylacetat. Its molecular formula is \( \text{C}_8\text{H}_8 \) and molecular weight is 138.16. Its melting point 67°-68°C. In the present work styrene is used to synthesise high molecular poly styrene sulphonic acid.

2.1.2.4. **Potassium per sulfate:** It is colourless, odourless crystal, decomposing more quickly at higher temperature and powerful oxidizing agent. Its molecular formula is \( \text{K}_2\text{S}_2\text{O}_8 \) and molecular weight is 270.32. In the present work potassium persulphate has been used as an oxidizing agent.

2.1.2.5. **Benzoyl peroxide:** Its molecular formula is \( \text{(C}_6\text{H}_5\text{CO}_2\text{)}_2\text{O}_2 \) and molecular weight is 242.23 and has melting point 103°-106°C. It is mainly used as a source of free radicals for industrial processes, oxidising agent in bleaching oils, flour, etc., catalyst in the plastics industry; initiator in polymerization and also acts as anionic flocculant agents.

In the present work benzoyl peroxide used as a free radical generator and chain transfer agent.

2.1.2.6. **Sodium metabisulfite:** Its molecular formula is \( \text{Na}_2\text{S}_2\text{O}_5 \) and molecular weight is 190.11. In the present work it has been employed for the preparation of
CHAPTER 2 Materials and Methods

sulphuric dioxide gas. Caution: Potential symptoms of over exposure are irritation of eyes, skin and mucous membrane

2.1.2.7. **Disodium hydrogen phosphate:** Disodium hydrogen phosphate or dibasic sodium phosphate, has the molecular formula Na$_2$HPO$_4$, mol weight is 141.98. It is anhydrous, hygroscopic powder, soluble in water.$^{73}$ It has been used as sequestrant, emulsifier and buffer in foods. As mordant in dyeing; forweihting silk; in tanning in the manufacture of enamels, ceramics, detergents$^{74}$ boiler compounds as fire proofing agent; in soldering and brazing instead of borax; as reagent and buffer in analytical chemistry.$^{73,75,76}$

In the present work disodium hydrogen phosphate is used for the preparation of buffer solutions.

2.1.2.8. **Sodium phosphate monobasic:** It’s molecular formula$^{73}$ is NaH$_2$PO$_4$, molecular weight is 119.98. It is a monohydrate, white, odourless slightly deliquescent crystal or granules. At 100°C loses all its water; when ignited it converts into metaphosphate. Freely soluble in water: partially soluble in alcohol. In the present work sodium phosphate used for the preparation of buffer solution.

2.1.2.9. **Lead subacetate:** ICUMSA recommends that the lead subacetate (basic lead acetate solution contain 10.0 ± 0.2 g pbvO per 100 ml with a density of 1.240 ± 0.002 g/ml. It’s molecular formula as Pob, soluble in water, insoluble in acetic acid. In the present work lead sub acetate acid used for the analysis of sugar juice purity.

2.1.2.10. **EDTA (Disodium salt of ethylene diamine tetra acetic acid):** It’s molecular formula is C$_{10}$H$_{12}$CaN$_2$ Na$_2$O$_8$, and molecular weight 374.27, it is white crystalline solid, soluble in water. In the present work disodium salt is used for the determined the calcium content of sugar of cane juice.
2.1.2.11. **Potassium Ferrocyanide:** Its molecular formula is K₄Fe(CN)₆.3H₂O, molecular weight 422.39, it is yellow solid, soluble in water. In the present work Ferrocyanide is used for the determined the calcium content of sugar of cane juice.

2.1.2.12. **Potassium iodide:** It's molecular formula is KI, molecular weight 166, white crystalline solid and soluble in water. In the present work potassium iodide is used for the determined the calcium content of sugar of cane juice.

2.1.2.13. **Erichrome Black:** Its molecular formula is C₂₀H₁₂N₃NaO₇, molecular weight 166, white crystalline solid and soluble in water. In the present work Erichrome black is used for the determined the calcium content of sugar of cane juice.

2.1.2.14. **Ammonium molybdate:** Its molecular formula is (NH₄)₆Mo₇O₂₄.4H₂O molecular weight 1235.86, soluble in water. In the present work ammonium molybdate is used for the determined of phosphate content in sugar cane juice.

2.1.2.15. **Stannous chloride:** Its molecular formula is SnCl₂.2H₂O, molecular weight is 225.65, it colourless powdered form soluble in water. In the present work stannous chloride is used for the determined of phosphate content in sugar cane juice.

2.1.2.16. **Copper sulphate:** Its molecular formula is CuSO₄.5H₂O molecular weight is 249.69, it is bluish green crystal, soluble in water. In the present work copper sulphate is used for the determined of reducing sugar in sugar cane juice.

2.1.2.17. **Sodium potassium tartrate:** Its molecular formula is C₄H₄KNaO₆.4H₂O, molecular weight is 282.22, it Rochelle salts, soluble in water. In the present work sodium potassium tartate is used for the determined of reducing sugar in sugar cane juice.
CHAPTER 2 Materials and Methods

2.1.2.18. Sodium Hydroxide: Its molecular formula is NaOH, molecular weight is 40.0, it is soluble in water. In the present work sodium hydroxide is used for the determination of reducing sugar in sugar cane juice.

2.1.2.19. Methylene Blue: Its molecular formula is C\textsubscript{16}H\textsubscript{8}CN\textsubscript{3}S.H\textsubscript{2}O, molecular weight is 319.85, in the present work methylene blue is used as indicator for the determination of reducing sugar in sugar cane juice.

Section -2: Sugar Cane Varieties:

2.2.0. Introduction: Karnataka is one of the major sugarcane growing states in India, where in sugarcane is growing in an area of about 2.41 lakh hectares with a total production of 21 million tones and with a productivity of 87 tonnes/hectare (swamy 1999). Although climatic factors are highly conducive for higher cane yield, but for quality it is found static (Sundra 1998), consequent to the fact that there was gap between cane growers and the sugar industry in selection and growing of sugar rich varieties.

There are four important varieties, Namely
1. Early maturing cane varieties
2. Mid-late maturing cane varieties and
3. Late maturing cane varieties.
4. Short duration varieties

2.2.1. Early Maturing Cane Varieties: Early maturing varieties attain 16% sucrose and 85% purity in 10 months of age under normal planting. They are ready for harvest at 10 to 11 months. Most of the early varieties are rich in sucrose, because of their relatively shorter vegetative growth phase. The yield is generally low.

Example: CoC-671, Co-6907, CoJ-64, CoC-92061
2.2.2. **Mid-Late Maturing Cane Varieties:** Mid-late maturing varieties\(^{11,78,79}\) attain 18% sucrose and 85% purity in 12 months. *Example:* Co-6304, Co-8021, Co-1148.

2.2.3. **Late Maturing Varieties:** Late maturing varieties\(^{11,78,79}\) attain 16% sucrose and 85% purity at the age of more than 12 months. Most of the late varieties are high yielding but generally have lower sucrose levels than the early and mid-late varieties. Most of the jaggery farmers prefer such varieties. *Example* Co-62175.

2.2.4. **Short Duration Varieties:** These varieties\(^{11,78,79}\) mature in about 8 months. These are considered important for multiple cropping to improve cane and sugar yields in short time. These varieties can be grown during the moisture favorable periods of the year. These cane can be planted with the onset of monsoon rains in June-July and harvested after 8 months in Feb-March before summer drought sets in. Most of the short duration varieties were found rich in sugar. *Example:* Co-8336, Co-8337, Co-8338, Co-8339, Co-8340, Co-8341.

2.2.5. **Common Varieties Grown in and around Mandya District:** In Mandya region Co-62175, Co-86032, Co-8371, Co-419 varieties are most commonly grown.

2.2.5.1. **Co-62175 (Co-951 X Co-419):** It is late maturing variety,\(^{11,78}\) they are thick cane and the yield potential is over 200 tonne/hectare. They possess moderate juice quality. Eye buds are prominent with a tendency to sprout *in situ*. It has a waxy coating and the rind is relatively soft. Less susceptible to smut, red rot, and *helminthosporium* mid late variety and matures in 13 months with 18-19% sucrose. It is a vigorous and good ratooner, good for jaggery, well adopted to excess moisture, and fiber rich. It is a most popular and commonly grown.
2.2.5.2. Co-8371 (Co 740 X Co 6806): It is mid-late maturing thick cane. It gives high yield with promisingly good quality, it has large internodes, low tillering; millable cane population is low, hence preferred under high density planting. It is hard to detash. The growth record is 180 tonnes/hectare yield with 20-19% sucrose. Its growth is promising in Kollapur region in Maharashtra. Although it is yield almost equal to Co-62175, but because of its better in quality made it popular in Karnataka.

2.2.5.3. Co-86032 (Co-62198 X CoC-671): It is mid-late maturing variety cane. It is attractive cane with high cane yield, its juice yield sucrose around 20%. It has a few hard deciduous leaf spinners and sparse splits. The cane is solid without pith formation. It is shy flowering to non-flowering maintains the quality for 2-3 months. It is good for jaggery making. It is an excellent ratooner and stands multi-ratooning under good management. It is resistant to smut and tolerant to salinity and moderately resistant to wilt. It is the most promising variety of the peninsular zone comprising Karnataka, Kerala, Tamil Nadu, Maharashtra, Gujarat, Madhya Pradesh, and interior Andhra Pradesh.

2.2.5.4. Co-419 (POJ 2878 X Co 290): It is late maturing cane, it is purple, vigorous thick cane, erect and non-lodging, it is which is high yielding and possess good juice quality 18-19% sucrose responds to high N, known as “first wonder cane of India” and was most predominant in the tropical states, currently grown in certain parts of Karnataka has undergone degeneration.

SECTION – 3 Instrumentation

A brief outline about the instruments used in the present investigation is presented in this section.
2.3.1. **Spectrophotometer:** Its used to analysis of colour intensity of the sugar cane juice

2.3.1.1. **Model, make- application:** Elico Spectrophotometer, Model SL 171 (Hyderabad, India) with 1 cm matched quartz cells

2.3.1.2. **Working principle:** Spectrophotometer methods are remarkable for their versatility, sensitivity and precision. Ultraviolet-Visible (UV-VIS) spectroscopy provides information about structure, formulation, and stability of the materials in solution. Both solids and liquid samples can be analyzed at concentrations in the parts per million (ppm) ranges. Absorption spectra are produced when ions or molecules absorb electromagnetic radiation in the ultraviolet or visible region. Spectrophotometric methods can be used for the determination of all the elements and a large number of organic compounds. A very extensive range of concentration may be covered from macro quantities (1 to 50 %) to traces (10^-8 to 10^-6 %). Until recently visible spectrophotometry was called colorimetry and even at present definitions such as colorimetric or absorptiometric methods are sometimes found in the literature, besides, the term spectrophotometric method in an identical sense.

The basis of spectrophotometric methods in the simple relationship between the absorption of radiation by a solution and the concentration of the coloured species in the solution.\(^4\)\(^-\)\(^6\) There is a close relationship between the colour of a substance and its electronic structure. A molecule or ion exhibits absorption in the visible or ultra violet region when the radiation (photons) causes an electronic transition in the molecule containing one or more chromophoric groups. The wide use of colorimetric and spectrophotometer methods in trace analysis is based on the possibility of converting the constituents to be determined into a substance whose solution is strongly coloured.
Such a solution shows differential absorption of light of different wave length of radiation by a system can be described by means of a plot of the absorption as a function of wavelength; such a graph is called as an absorption spectrum. This usually shows one or two pronounced maxima or minima, but sometimes that may be more complex. The capability and capacity for exhibiting characteristic absorptive for specific wavelengths for incident radiant energy is directly related to the composition and concentration of the absorbing species.

The fundamental law governing the absorption of all types of electromagnetic radiation is known as the Lambert-Beer law or Beer's law. This basic law of spectrophotometry is given by the equation:

\[
\log \frac{I_o}{I} = \log \frac{1}{T} = \varepsilon bc = A, \text{ absorbance}
\]  

(1)

Where \( T = \text{transmittance} = \frac{I}{I_o} \)
\( I_o = \text{radiant power of the beam striking the sample}; \)
\( I = \text{radiant power of the beam transmitted by the sample}; \)
\( C = \text{concentration of the absorbing constituent of the sample in mol \( l^{-1} \);} \)
\( \varepsilon = \text{a constant known as molar extinction coefficient, whose value depends on the identity of the absorbing species, the wavelength of light, the nature of the solvent, the temperature, etc., units of } \varepsilon \text{ are } 1 \text{ mol}^{-1} \text{ cm}^{1}. \)

The equation (1) shows that the absorbance of a solution is directly proportional to the concentration of the absorbing species. It is evident that a plot of absorbance at constant \( 'b' \) against concentration gives a straight line if Beer's law is followed.

Beer's law is successful in describing the absorption behavior of dilute solution only; in this case it is a limiting law. At high concentrations, the average distance between solute molecules (or ions) is diminished to the point where each affects the charge distribution of its neighbors. Apparent chemical deviations of Beer's law are frequently encountered as a consequence of association, dissociation or reaction of the
absorbing species with the solvent. Beer's law is valid only when monochromatic radiation is employed which is another limitation of the law. However, it is desirable that the solution should follow Beer's law up to a concentration corresponding to absorbance of 1.0

2.3.1.3. Sensitivity of Spectrophotometric Methods: Sensitivity refers to the slope of a calibration curve, but is frequently used to mean the least determinable concentration or amount of the species of interest. The objective numerical expression of the sensitivity of spectrophotometric methods is the molar absorptivity ($\varepsilon$) at the wavelength of maximum absorbance of the coloured species.

$$\varepsilon = \frac{A}{bc}$$

For more sensitive spectrophotomeric methods "C" is greater than $1 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ and for the to less sensitive methods it would correspond to the value of $1 \times 10^3$ l mol$^{-1}$ cm$^{-1}$. The molar absorptivity cannot exceed $1.5 \times 10^5$ according to quantum theory. The sensitivities of spectrophotometric methods for compounds with similar molecular weights can be compared in terms of molar absorptivity values. In order to give a comparison that is independent of molecular weights, the sensitivity is often expressed in terms of the expression called sensitivity index, given by Sandell$^{87}$ which indicates the number of micrograms of the determinant per mol of a solution having an absorbance of 0.001, for a path length of 1 cm. The sensitivity is expressed as $\mu$g cm$^{-2}$.

In the determination of $\varepsilon$, absorbance measured should be within the range over which the coloured system confirms Beer's law and within the range of values for the measurement error is minimal. In addition, the slit width used is of importance.
2.3.2. **Polarimeter:** The instrument used for quantitative estimation of sugar by optical methods.

2.3.2.1. **Polarimeter Light:** A beam of ordinary light is made up of vibrations that proceed in an infinite number of planes. By means of various optical devices, it is possible to cause a beam of light to vibrate in one plane only. Such light is said to be polarized or, more specifically, plane polarized, and the plane in which it vibrates is called the plane of polarization.

Many substances, including solution of sucrose and other sugars, have the power to rotate the plane of polarization. The angle through which the plane of polarization is rotated may be measured by suitable optical instruments, and the analysis of sugars by optical methods employs the measurement of this rotation. Sucrose rotates the plane of polarization to the right, and hence called *dextrorotatory* sugar. Dextrose also rotates the plane to the right. Laevulose rotates the plane to the left and is a *levorotatory*, or a left-hand sugar.

Quartz is of two kinds, right and left hand, and is used in sugar polarimetry to compensate for the rotation caused by the sugar solution.

2.3.2.2. **Specific Rotary Power:** The power to rotate the plane of polarization differs from different sugars. The specific rotation, is expressed by the relation.

\[
[\alpha]_\lambda = \frac{100\alpha}{C} = \frac{100\alpha}{Bd} = \frac{100\alpha}{Cl} = \frac{100\alpha}{Bdl}
\]

Where \( \alpha = \text{me} \)
\( C = \text{concentration of sugar (g/100ml of solution)} \)
\( B = \text{Brix of solution (g/ of sugar/g of solution)} \)
\( d = \text{density of solution (g of solution/ml of solution)} \)
\( l = \text{Cell length (dm)} \)
CHAPTER 2

Materials and Methods

\[ t = \text{temperature}^\circ\text{C} \]
\[ \lambda = \text{wavelength nm} \]

Since the specific rotation depends on the wavelength of light employed and the temperature at which it is taken, these variables must be specified. The bright yellow line of sodium light, known as the D line, is the light sometimes used and the standard temperature is 20°C, the symbol for specific rotary power under these conditions being \([\alpha]^{20}_{D}\). Another useful light source is the blue-green line in the mercury arc at 546 nm. With the advances in laser technology, these beams will probably soon be used as a light source of superior definition. An easily obtained wavelength in the red will most likely be used for polarimetry.

2.3.2.4. Principle: When a ray of polarized light is passed through a solution of any given sugar, the amount of rotation varies with the concentration of the solution, the length of the cell, the wavelength of the light, and the temperature. By having a fixed length of cell, standard temperature, standard weight and standard volume, and standard light source, the rotation becomes a function of the concentration of the sugar in the solution. Measuring the rotation under these fixed standard conditions determines the concentration of the sugar present, subject to the previously noted conditions for mixtures of various sugars. That is, the rotation is an accurate measure of the sucrose concentration if no other optically active substances are present.

2.3.3. Brix spindles: Instruments specially graduated for sugar work are sometimes termed "saccharometers," but possible confusion with "saccharimeters" makes the word objectionable. Figure ( ) show a high-grade Brix hydrometer with thermometer and temperature correction enclosed. In this type of instrument, the temperature is the same as that at which the Brix is taken, and reference to tables is obviated. The stems of the instruments should be of small diameter, and the range
CHAPTER 2 Materials and Methods

should not exceed 6° graduated in tenths. Spindles for use by factory operators omit the thermometer. The standard temperature approved by all governing bodies is 20°C, and observations made at other temperature also as per standard reference table.

Many tropical countries use the hydrometers standardized at 27.5°C, and ISSCT recommends this standard temperature for all tropical countries. However, air conditioning has been introduced into most sugar laboratories; work at 20°C now supersedes 27.5°C.

2.3.3.1. Method of Using Hydrometer: The sugar solution was allow to stand until all air bubbles have risen to the surface. Suction, applied by placing a large rubber stopper fitted with a glass tube connected with a vacuum line, assists in the removal of air. Float the clean, dry spindle in the deaerated sugar solution, lowering carefully into the liquid so that the stem is wet for 2 or 3 mm above the point where it comes to rest. Allow sufficient time for the hydrometer to reach the same temperature as the solution; then read the point at the level of the liquid. For dark solution, it is estimate from the level of the liquid temperature of the solution was recorded on the enclosed thermometer and correction was applied. This spindle should not touch the sides of the cylinder when the reading is taken, and for accurate work temperatures should be near the standard, 20°C.

2.3.3.2. Testing Hydrometers: All hydrometers for laboratory control work require testing before being put to use. The best grades carry a maker’s serial number for identification and for maintaining a record of corrections found. The test solution is pure sucrose on which the percentage of sugar has been determined by the polariscope, by a high-precision refractometer, or less frequently by weighing the required amount.
CHAPTER 2

Materials and Methods

of sugar and making to volume in a volumetric flask. High-purity refined sugar, such as cubes or large-grain confectioners, serves for general test purposes.

In laboratories having air-conditioned polariscope room, spindles may be tested at or near 20°C and again at higher laboratory working temperatures. For instruments with enclosed thermometers, which have several advantages, the calibration checks both the hydrometer and the thermometer.

2.3.4. **pH Meter:** The colorimetric determination of pH has been largely superseded in many areas by the use of pH meters and automatic electrometric pH recorders; but for rapid routine comparison work, especially on light-colored solutions, many retain the colorimetric methods. The colorimetric test depends on certain indicators. Ready-made color standards in sealed ampoules are available that are guaranteed constant for an indefinite period. Nevertheless, periodic checks on all colorimetric standards should be made electrometrically.

**Indicators.** The indicators for use in routine raw sugar work are as follows:

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Solution</th>
<th>pH Range</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromthymol blue</td>
<td>0.04%</td>
<td>6.0-7.6</td>
<td>Yellow-blue</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.02%</td>
<td>6.8-8.4</td>
<td>Yellow-red</td>
</tr>
<tr>
<td>Cresol red</td>
<td>0.02%</td>
<td>7.2-8.8</td>
<td>Yellow-red</td>
</tr>
<tr>
<td>Congo red</td>
<td></td>
<td>2.8-4.8</td>
<td>Blue-red</td>
</tr>
<tr>
<td>Methyl red</td>
<td></td>
<td>3.8-6.1</td>
<td>Red-yellow</td>
</tr>
<tr>
<td>Phenolphthalein</td>
<td></td>
<td>8.0-9.6</td>
<td>Colorless-red</td>
</tr>
<tr>
<td>Thymolphthalein</td>
<td></td>
<td>10.2-11.7</td>
<td>Colorless-blue</td>
</tr>
</tbody>
</table>

2.3.4.1. **Electrometric determination of pH:** The development of electrometric pH measurements has resulted from the introduction of the glass electrode. Assemblies suitable for both laboratory and plant use are available in all over the globe. Such equipment is rugged, simple to operate, and reasonably priced; thus, laboratory technician now determine pH electrometrically more conveniently with much greater
accuracy and speed than colorimetric tests. The electrometric control of pH for both cane juice and refinery clarification is standard practice as an industrial operation.

2.3.4.2. **Principle:** Electrometric pH measurement depends on the voltage generated by an electrode system consisting of a glass electrode with a detecting bulb of H\(^+\) sensitive glass, immersed in the test solution, and a reference electrode, generally incorporating a calomel half-cell and saturated potassium chloride bridge. Silver-silver chloride reference electrodes with potassium chloride as the electrolyte are also employed. The liquid junction is either a porous plus or a ground-glass-sleeve type to permit slow leakage of the potassium chloride solution into the sample. Double-junction reference electrodes are now available for use when KCl contamination is undesirable. Combination electrodes are available for use with small samples.

In recent years highly dependable instruments were designed. These modern instruments have a temperatures of reading in both mill volts and pH, divisions to 0.1 pH on mirror-backed scale, automatic or manual temperature compensator temperature ranges of 0-100°C, compensation for variations in line voltage, and small zero drift for normal working periods. Most models provide a dust-free cover that excludes moisture, even in tropical countries. All models provide for a solution holder at the side of the instrument into which the electrode assembly dips when making the determination.

2.3.4.3. **Method of Operation:** The electrode assembly should be checked at least daily with buffer solution of 7.0 pH. All samples for ordinary control work should be cooled to room temperature. Temperature compensation is built in on most pH meters; that correction is advisable. The electrode assembly is rinsed thoroughly with the test solution and immersed in the solution to sufficient depth to cover the bubbles of the electrodes. The temperature compensator is adjusted to the temperature of the solution.
and the pH is read. The pH will drift for a period of time that varies directly with the density of the solution. A set time must be allowed to elapse before reading is taken. ICUMSA recommends that following immersion of the electrode in the sugar solution the reading should be taken after a minimum of 5 min.

The sample should be tested without dilution if possible. For raw sugars, Hawaii\textsuperscript{15} recommends dissolving two parts sugars in one part of less of water. For molasses, determination may be made at 1:1 dilution. For white sugar ICUMSA recommends the direct measurement at 50 Brix and 20°C.

2.3.5. Refractometers.

2.3.5.1. Refractive Index: When a beam of light passes at an angle from one medium, (air) into another medium (water) the rays of light are bent or refracted. The amount that the light is bent is proportional to the velocity of light in the two media. The measure of the amount that the light is bent is termed the index of refraction $\mu$: The refractive index.

$$\mu = \frac{\sin i}{\sin r}$$

Which varies with temperature, wavelength and concentration of the solution. Obviously, the refractive index of a pure sucrose solution is a measure of the sucrose content, and just as the Brix is extended for convenience to indicate solids other than pure sucrose in solutions.

With high-test solutions, the refractometer is affected only by the solids in solution; therefore, if the material under examination contains insoluble matter, this is not included in the estimate of the solids. Within its limitations, the refractometer is
capable of giving accurate measurement of the solids content. With low-grade materials such as molasses, in soluble material does not affect the refractometer reading.

Many refineries and factories work with refractometer Brix in preference to hydrometer readings. Refractometer Brix differs from degrees Brix by hydrometer; thus, control figures (example - boiling house efficiency) are higher than when degree Brix is employed.

The Abbe refractometer are most commonly used in sugar work, for measuring transmitted light for samples that shows low absorption and to measure reflective weight for lightly absorbent samples.

2.3.5.2. Hand refractometers: The hand refractometers read directly percent solids to about 0.5% sugar. Modern models have greater accuracy and enclosed temperature-correction scale. These instruments used to check the maturity of cane and beets in the field.

SECTION - 4: Experimental Methods

The present project work a modest attempt has been made to synthesize different experimental techniques from which one can clearly and easily understand the effect of different molecular weight and molecular structure of polymers.

The crushed cane juice\textsuperscript{82} (cold condition) analysis is involved measurement of Brix (Bx) and the total polarizing substance (Pol), i.e. the purity of the mixed juice, then the crushed juice using clarification of the juice by using the chemical clarificants like, primary flocculants are milk of lime and sulphur dioxide and phosphate, secondary flocculants are water soluble polyelectrolytes\textsuperscript{83} i.e., synthesized compounds. The clarified juice analyzed for mud settling, calcium content, reducing sugar, phosphate content, colour of the sugar cane juice and turbidity of the clarified juice.
CHAPTER 2 Materials and Methods

The present study deals with various aspects of cane varieties growing in Mandya zone. A special emphasis to their chemical composition, response to clarification, which purity rise, settling characteristic, extent of mud settling, floating scum nature includes colouring matter and the pH. Sensitivity of colourants were based on the results of the analysis, clarification properties of juice of individual cane variety performance at laboratory scale.

2.4.1. Mud Settling: For the laboratory tests as well as for process observations the settling tests we are conducted general producer for settling tests adopted is as follows

1. The different samples were collected at the appropriate source point and terms
2. The juice was heated for ensuring the uniform conditions for all the tests as temperature varies generally from sample site to site and laboratory in order to study the results under optimum conditions

The extracted juice is transferred into 6 beakers of 1000ml capacity. The juice in all six beakers were heated on a hot plate. The juice heated to 65\(^\circ\)C then milk of lime (12 Beaum, 1.8\% V/V) and sulphur dioxide were added subsequently heated to 80\(^\circ\)C. First beaker was treated as blank, different doses of flocculating agents corresponding in the range 2 to 10 ppm were added drop by drop with constant swirling to the remaining five beakers. After this contents of the beakers were quickly transferred to a measuring cylinder. The mud volume was measured for every 30 seconds up to 30 minutes. The settling rate were measured and the clear juice obtained was tested for purity, calcium content and phosphate content.

The initial settling rates were determined in each case and a plot of initial settling rate against % hydrolysis of the flocculant, was drawn. The optimum flocculant required to produce lower turbidity and high initial settling rat, were determined. All the analytical procedure used are in accordance with the norms prescribed by ICUMSA.
2.4.2. Determination of purity of cane juice:

_Brix:_ Juice is filtered to overflow in a cylinder placed perfectly vertical, then allowed the air to escape by standing for 20 minutes. Alternatively, the air is be removed by applying vacuum, gradually lower the standardised Brix spindle of the approximate range in position. When the spindle becomes steady, by which time it attained the juice temperature, the readings of the spindle were recorded. The temperature of the juice was also recorded. The corrected brix of the juice is given by the reading on the hydrometer from the standard chart.

_Pol:_ 200 ml of juice was transferred into a 300 ml cleansed Erlenmeyer flask, to this 2-3g of Horne’s dry subacetate of lead (about one gram per 100 ml of juice) was added with constant swirling, then filtered using filter paper and dry funnel. The funnel was covered with a watch glass during filtration to avoid the entrap of air from the atmosphere. The filtrate was collected in a clean dry beaker, first few milliliters of filtrate was rejected. The remaining filtrate was taken in the pol tube, and their it was placed in the polarimeter (only after the absence of air bubbles in the was observed). The experimental results were recorded. The average of the reading were calculated from which the pol percent juice was determined with reference to Schmitz table. The purity of the juice was determined using the relation.

\[
Purity\ of\ juice = \frac{Pol\ %}{Corrected\ brix} \times 100
\]

2.4.3. Measurement of pH: Clean and dry glasswares were used, the solution was prepared as per the standard known method. PH meter was calibrated as per the instrument manual. PH of the sample solutions at 30°C.
2.4.4. **Measurement of Colour:** The colour of the sugar was analysed by the standard GS 2/3 –10 method.

The sample juice solution was taken in a perfect cleaned beaker; the pH of the solution was measured. The solution was filtered through 0.45 micron pore size membrane filter paper under the vacuum condition. The filtrate was allowed for 20-25 minutes for deaeration. After deaeration the wavelength of absorption of the sample solution was determined at a wave length of 420 nm using 1 cm path length cuvettes. The same procedure adopted for multiple samples.

2.4.5. **Determination of Phosphate content:** 100 ml of juice is taken in a conical flask. Sample juice solution adjust the pH 4.0 ± 0.5 with dilute H₂SO₄ (1:10). 1 g of dry lead sub acetate was added with constant swirling. Then filtered the juice using whatman filter paper No-41 and 10 ml of this clarified juice is transferred into 50 ml volumetric flask, 10 ml acid molybdate solution (16.6g ammonium molybdate tetrahydrate + 96.0 ml of con. H₂SO₄ and made the vol 1000 ml) and six drops of saturated stannous chloride solution were added and finally the solution is made to 100 ml. The absorption is measured in a spectrophotometer and was compared characteristic blue colour of the standard in a spectrophotometer. The colour faded after 10-12 minutes. Retension of blue colour was observed with the stannous chloride for another 10 to 12 minutes.

2.4.6. **Determination of Calcium Content by EDTA Method:** 150 ml of juice is taken in a conical flask 0.5g lead sub acetate is added and 60 ml of this clarified juice is transferred into dry conical flask, powered potassium ferro cyanide is added is small quantities till there is no further precipitation. The juice is shaken well and filtered, filtrate is tested for the absence of lead with potassium iodide, 10 ml of this filtrate is
CHAPTER 2 Materials and Methods

pipette out into a conical flask, 5 to 6 drops of liquor ammonia and add two drops of Erichrome blue as an indicator. The content were titrate against standard EDTA solution, till the end point is reached. The amount of Ca$^{2+}$ is determined.

2.4.7. Determination of reducing sugar in the juice sample: The juice solution of 125 – 200 ml of reducing sugar is prepared. The juice sample that contains no preservative is filtered directly through dry kieselghur and no lead or other clarification is used. Samples preserved with mercuric chloride may also be treated this way. But juices containing formaldehyde should never be used for invert determination since formaldehyde reduces copper. Before adding the kieselghur, dry sodium oxalate (about 0.25g for each 100 ml of juice) should be added to remove lime salt.

Titration: Exactly 10 cm$^3$ of fehling's solution was taken in 250 cm$^3$ Erlenmeyer flask, and heated the solution neatly to boil over a wire gauge, to the hot solution 3-5 drops of methylene blue indicator was added the contents of the flask were titrated against the sugar solution with constant shaking. Titration is carried out maintaining the solution hot and continued till the disappears of blue colour and red precipitate of cuprous oxide is obtained.

2.4.8. Methods of Preparation of Homogenous Flocculent Solution: Homogenous solutions of the flocculent in hot water is prepared. It is found that 0.75 to 1.0 gram of the flocculent diluted in one liter of water is suitable for making an ideal solution. The solution of this concentration is easily spread in missed juice on addition. If the water used for dilution is lesser than this proportion. The jelly like translucent lumps is produced and spread allover the liquid. If this jelly mass once formed. It is not easily made in to a uniform solution even on stirring with any amount of water or in mixed juice after addition.
CHAPTER 2 Materials and Methods

The simultaneous stirring effect is to be made during the dilution of the flocculent. If either mechanical or manual stirring is done after dilution gives the chances of the formation of this jelly mass.

A slow stream of the solid flocculent is added suitably on the forced stream of water from the narrow pipe so that the required quantity of the flocculent is added continually till the water is filled up to 8% of the total capacity.

The tank capacity is selected so that the entire shift dose is diluted once in a shift with the above concentration. And it is fed into the mixed juice uniformly by controlling the value such that $\frac{1}{8}$ of the graduated division falls per hour.