CHAPTER-3

MATERIALS AND EXPERIMENTAL METHODS

In this chapter materials and experimental methods used are described. It includes materials used in the experiments, the analytical procedures used for determining naringin concentration and pectin content, characterization of pectin, the process for preparation kinnow peel boiled water and subsequently adsorption and desorption procedures to obtain naringin and pectin along with flow charts are given.

3.1 Materials

3.1.1. Adsorbents (Resin)

Macroporous, nonionic, hydrophobic, cross-linked polymeric adsorbent resins INDION PA-500 and INDION PA-800 (Ion Exchange India Ltd.) have been used; properties of both adsorbent resins have been given in Appendix Table A1.

3.1.2. Chemicals/various solutions

Chemicals used in the experiments were of analytical reagent grade. Naringin ($C_{27}H_{32}O_{14}$) and Galacturonic acid were purchased from Sigma-Aldrich Chemicals Pvt. Ltd. India, Diethylene glycol ($C_4H_{10}O_3$), Citric acid ($C_6H_8O_7$), Ethanol (C_2H_5OH), Hydrochloric acid (HCl), Sulphuric acid (H_2SO_4), and Carbazole reagent were purchased from Merck Specialities Pvt. Ltd. India, Sodium hydroxide (NaOH), Potassium metabisulfite ($K_2S_2O_5$) were purchased from Qlualigens Fine Chemicals Pvt. Ltd. India, Distilled water was used for preparing all the solutions.

3.1.3. Kinnow peels

Kinnow peels used in the experiment were collected from the market in Varanasi from juice vendors during December 2011 to January 2012, December 2012 to January 2013 and

December 2013 to January 2014. These are mentioned in the text as peels of years 2012, 2013 and 2014 respectively. Preharvest dropped kinnow peels used in the experiments were obtained from fruits collected from Abohar (Punjab), India during the months October of the year 2012 and procured from Sriganga Nagar (Rajasthan), India in 2013. For dry peel experiments, fresh peels collected from fruit vendors was sized to less than 2 cm and were dried in a circulatory tray dryer at 60^{o}C for 24 h.

3.2. Analytical Procedures

(A) Naringin (Davis test)

In the present study, the naringin concentration was determined using the Davis test (1947) with a spectrophotometer (Elico double beam SL 210 UV-VIS). It is described below.

Preparation of solutions:

(i) Diethylene glycol solution (90%) was prepared by adding 100 ml of distilled water to 900 ml of diethylene glycol and mixed thoroughly to make 1000 ml of solution (ii) Sodium hydroxide solution (4N) was prepared by dissolving 16 g of NaOH in distilled water and made up to 100 ml.

A stock solution of concentration 1000 ppm (1 kg/ m^3) was prepared by dissolving 100 mg of naringin in 80 ml of warm distilled water. After that, contents were brought to room temperature and makeup exactly 100 ml. To make 125, 250, 500, and 750 ppm standard solutions, the stock solution was diluted with distilled water.

Procedure:

In this method. 0.1 ml of the sample was added to 10 ml of 90% diethylene glycol and 0.1 ml of 4N NaOH solution. After mixing, the solution was kept for 10 minutes for yellow color development. The absorbance of the colored solution was measured using spectrophotometer at 420 nm against distilled water blank. The calibration graph (absorbance vs naringin

concentration) was drawn by measuring the absorbance of standard solutions of naringin, and the curve was used to measure the naringin content in the unknown sample. The calibration curve for determination of naringin is shown Figure 3.1.

In desorption studies, 1.0 ml of the sample (ethanol-naringin solution) was taken, and ethanol was evaporated to get dried sample. This dried sample was dissolved in 5.0 ml of distilled water, and naringin concentration was measured by using Davis test as discussed above. The naringin concentration in the desorbed sample was obtained from the amount of naringin and volume of alcohol solution.

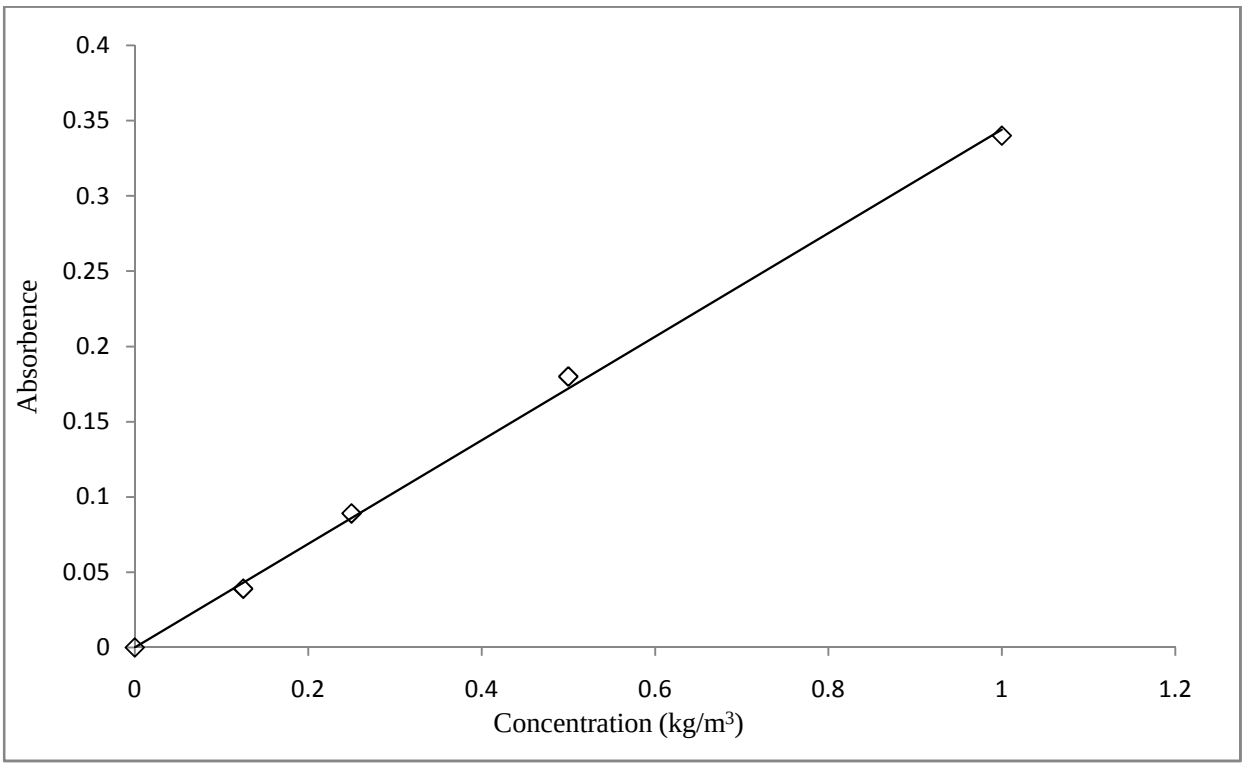

Figure 3.1: Calibration curve for determination of naringin

(B) Pectin

Colorimetric Method (Ranganna, 2005) was used for analysis of pectin in the samples. It is based on the reaction of the galacturonic acid, the basic structural unit of pectin molecule,

with carbazole in the presence of H_2SO_4 and measurement of the colour at 525 nm. The results of these experiments expressed as anhydrogalacturonic acid (AuA).

Procedure:

Unknown pectin sample preparation:

100 mg of pectin sample was dissolved in 100 ml of 0.05N NaOH solution. The solution was allowed to stand for 30 min to de-esterify the pectin. The 2 ml of this solution was diluted to 100 ml with distilled water. However, in the case of KPBW, 2 ml of this solution were taken, and 0.05 N NaOH was added to make 100 ml.

Blank Preparation: To prepare blank, to the 2 ml double distilled water and the chemicals are added in the same manner except for 1 ml of 100% ethanol in the place of carbazole reagent. Exactly after adding acid, the transmittance was set 100% with the blank.

Standard pectin solution preparation:

Accurately weighed 109.3 mg of galacturonic acid monohydrate (MW. 212) was transferred to a 1000 ml volumetric flask. 10 ml of 0.05N NaOH was added, and it was diluted up to the volume. The contents were mixed thoroughly and were allowed the solution to stand overnight. One ml of this standard solution contained 100 µg of AuA. To make different concentrated solutions, the standard solution was diluted with distilled water.

Two ml of the prepared pectin solution was transferred into tubes using a pipette, and 1.0 ml of carbazole reagent was added to get the white flocculent precipitate. To this precipitate 12 ml of conc. H_2SO_4 was added with constant agitation. The tubes were closed and allowed to stand for 10 min for color (dark purple) development.

Two (2.0) ml of standard solutions were used for color development as per the procedure described above. The absorbance of the colored solution was measured using spectrophotometer at 525 nm. A calibration curve was drawn by plotting the absorbance against the concentration of anhydrogalacturonic acid for known samples. The concentrations

of the unknown solution were determined by using calibration curve. The calibration curve for determination of pectin is shown in Figure 3.2.

This method was used only for measuring the concentration of pectin as AuA in KPBW and obtained pectin for knowing the percentage recovery of pectin.

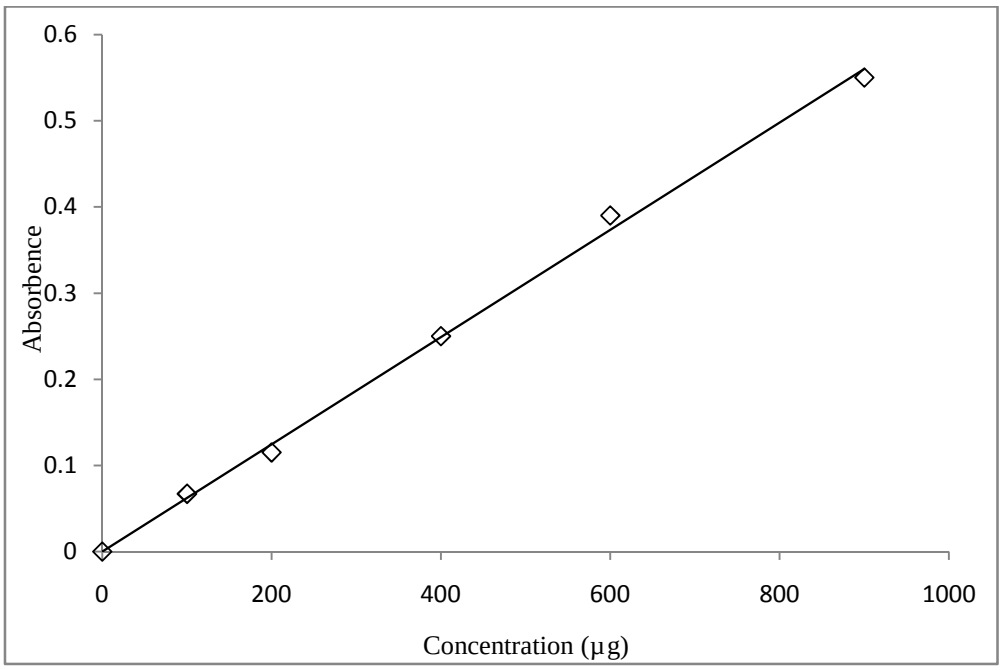

Figure 3.2: Standard curve for determination of pectin

3.3. Experimental Procedures

3.3.1. Preparation of Kinnow peel boiled water (KPBW)

The peels were cut in smaller size pieces about 2 cm, after that combined with four times of water (by weight) in case of fresh and dropped peels whereas 0.300 kg in the case of dried peels was added in 4 liters of water. The contents were boiled for 60 min, and peel solution was filtered through muslin cloth, and the extract set aside. The residue was mixed with another four parts of water (by weight) and boiled for another 60 min, followed by filtration with a muslin cloth. It was observed that for one kg of fresh peels about 0.300±0.015 kg dry peel is obtained.

The same step was repeated for one more time. The three extracts of peel samples were brought to room temperature and combined. Potassium metabisulfite (KMS) was added at a rate 2 g/l (2 kg/m^3) to prevent microorganism growth in the peel boiled water. This extract named as Kinnow peel boiled water (KPBW) and used for adsorption experiments and recovery of naringin and pectin.

3.3.2. Extraction of Naringin and Pectin

(A) Naringin

A glass column with ID 14 mm was used for the extraction of naringin; about 150 g wet conditioned resin (conditioning procedure is described in section 3.4.1) was transferred to the column. The column containing resin was treated with 0.4% aqueous citric acid solution, about two liters of the solution was passed through resin at a flow rate of 2 ml/min (3.3×10^{-8} m^3/s). The conditioned resin was treated with citric acid, to prevent the protein deposition on the resin in the column (Shaw, 1990). The kinnow peel boiled water (KPBW) was allowed to pass through the bed of resin at a constant flow rate of 2 ml/min (3.3×10^{-8} m^3/s) until the naringin concentration of the outgoing KPBW was equal to inlet KPBW solution (i.e., the resin is saturated with naringin). The remaining KPBW in the column was drained. Pure ethanol was passed through the resin (saturated with naringin), for desorption of naringin at a flow rate of 2 ml/min (3.3×10^{-8} m^3/s) of outgoing solution. The solution of naringin and ethanol was collected.

The desorbed naringin-ethanol solution which was 1500-2000 ml was distilled by using simple distillation up to a volume 150 ml. After, that the concentrated ethanol-naringin solution was filtered using Whatman filter paper no. 42. The filtrate solution was passed through membrane syringe filter of size 0.45 µm, and the filtrate was evaporated by heating till disappearance of liquid and after that put in an oven at 60^{o}C to recover naringin solid. The naringin extraction flowchart is shown in Figure 3.3.

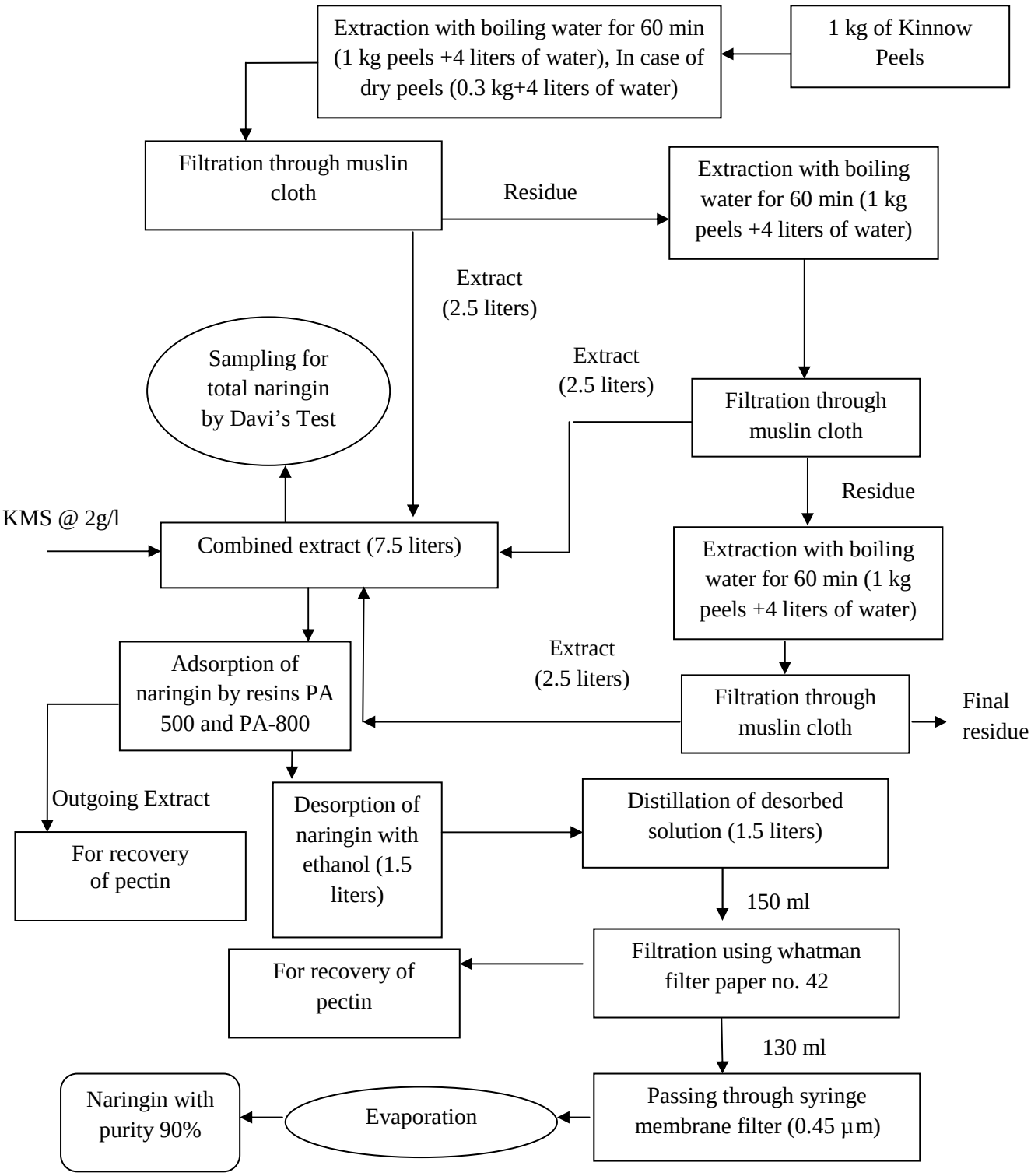

Figure 3.3: Naringin extraction process flow chart

(B) Pectin

The method for extraction of pectin is presented in Figure 3.4. The Kinnow peel boiled water collected after resin treatment was used for recovery of pectin. The pH of the above was adjusted to 2.2 with HCl. The contents were heated in a boiling water bath at 80^{o}C temperature for 15 min. The contents were cooled to room temperature and centrifuged at 5000 rpm for 35 min. The pH of above was adjusted to 7 with 4N NaOH. Ethanol was added to above solution at a final concentration of 60% (v/v). The precipitate obtained was separated and collected by centrifugation. The precipitate was washed twice with 80% ethanol to get crude pectin. This was air dried at 60^{o}C to get pectin.

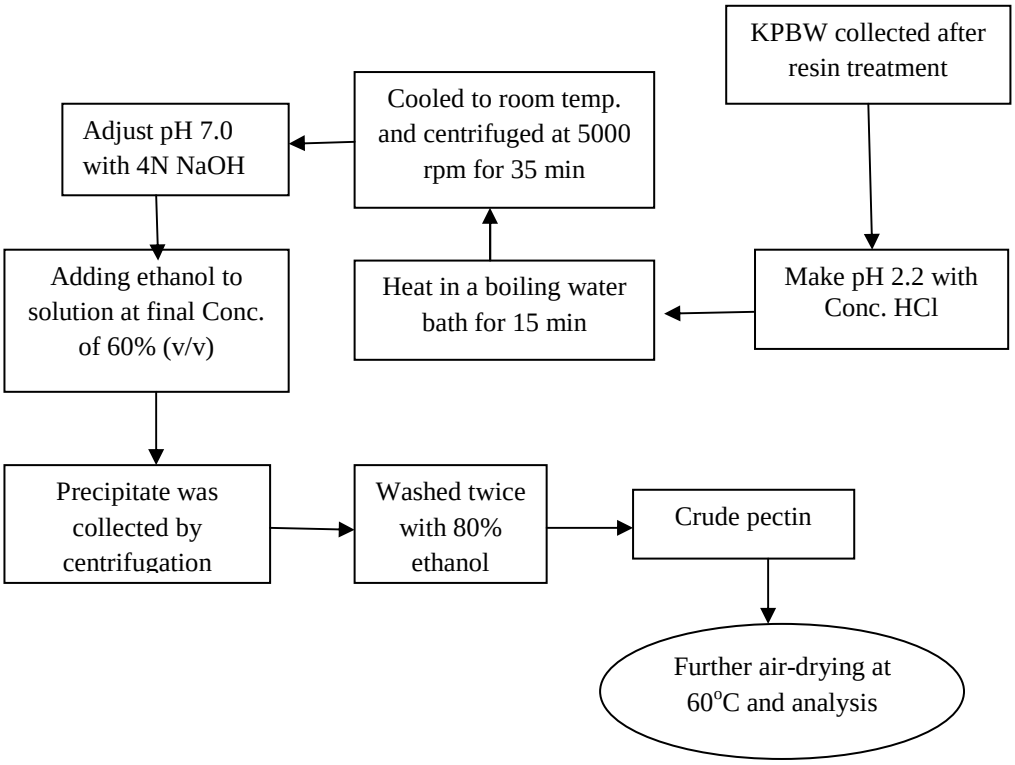

Figure 3.4: Pectin extraction process

3.4. Adsorption Experimental Procedures

3.4.1. Conditioning of Resin

Industrial resins contain a little organic and inorganic impurities. When the resin is contacted with the liquid solution, these impurities go into the solution and affect its purity. For that reason resin procured from the manufacturer must be conditioned before systematic experimental work is carried out. It has been carried in the same manner as mentioned by Singh *et al*. 2008.

The experimental setup for conditioning of resin is shown in Figure 3.5. For the conditioning of the resin procured from the manufacturer, it was transferred to a glass column. The backwash of resin bed was carried with distilled water to remove any suspended impurities. NaOH solution (1N) was passed through the fixed bed. The flow rate was corresponding to 4 bed volumes per hour. In all 20 bed volumes of NaOH were passed during 5 h. Backwash with distilled water was done to remove all traces of NaOH solution. After this, the bed was treated with 1N HCl solution, exactly in the same manner as done with NaOH. Backwashing with distilled water was done to remove all traces of HCl solution. The cycle was repeated 2 times to get conditioned resin.

Storage of Conditioned Resin:

The conditioned resin was stored in distilled water. When the resin was treated with 0.4% citric acid; it was stored in 0.4% citric acid solution. In all the experiments citric acid treated resins were used.

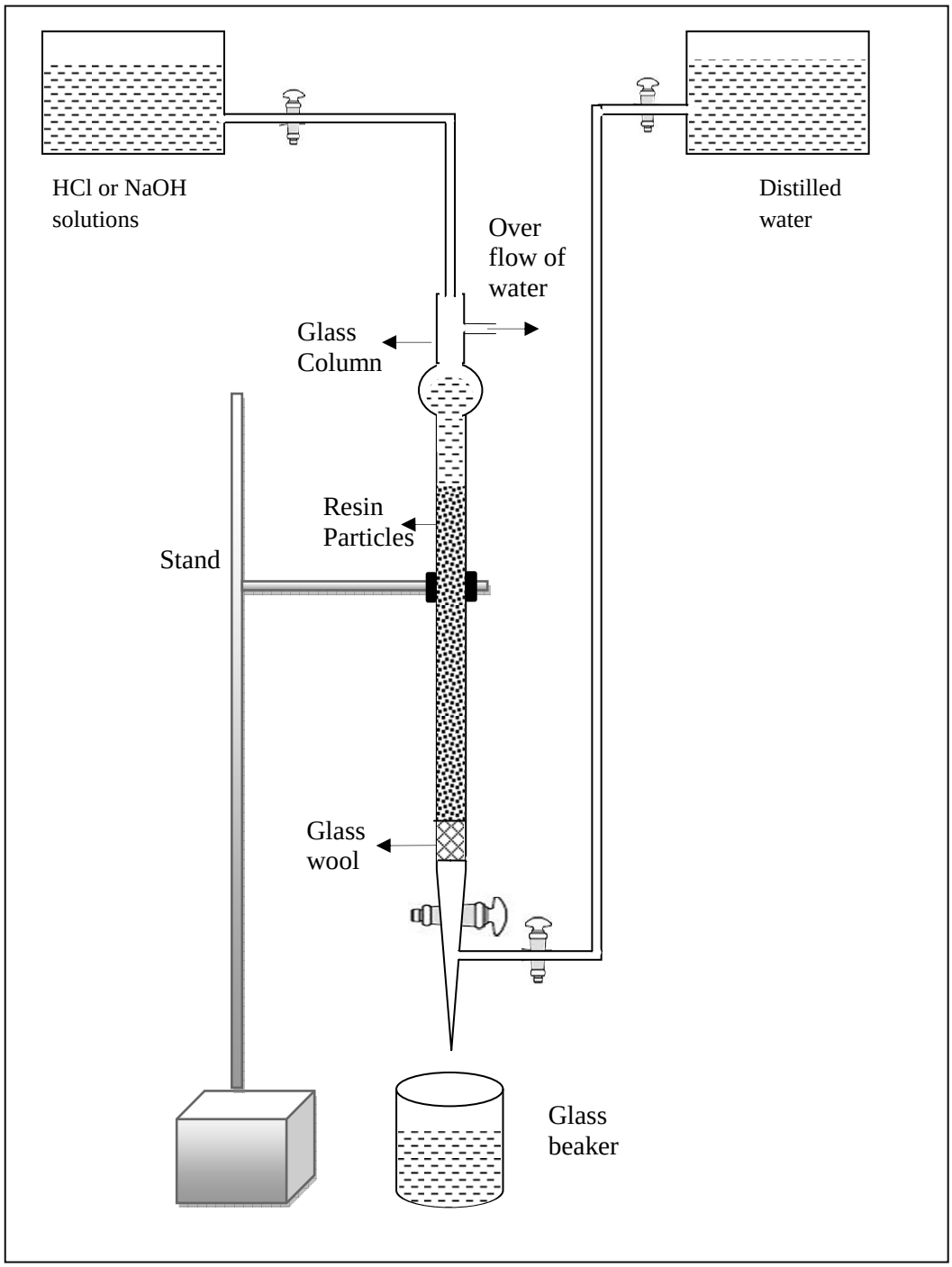

Figure 3.5: Setup for conditioning of resin

3.4.2. Solid content of Resin

A sample of the resin was taken, and the adhering surface moisture was removed by pressing it gently between the folds of filter paper. It has been termed as filtered paper dried resin (FPD). A part of it (1 to 2 g) was transferred to the oven and dried at 105^{o}C for 48 h,

followed by cooling in a desiccator. The dried resin was called as oven dried resin (ODR). The solid content is defined as the ratio of the weight of ODR resin to FPD resin. During every adsorption-desorption experiments, the solid content was determined for a specific specimen of the FPD resin. The number of adsorption sites depends on the area per unit dry mass. Since the mass of dry resin will give the actual representation of mass of adsorbent. Therefore, solid content is helpful in determining the actual weight of the resin used in adsorption experiments from FPD resin mass used. For the same specimen of FPD sample, the solid content was determined in duplicate and ensures that it was reproducible within experimental limits. Though, the experiments were carried out with FPD resin; the calculations were done on the basis of ODR as per standard practice.

$$\text{Solid content (s)} = \frac{Wt.\ of\ \ O.D.R\ resin}{Wt.\ of\ \ F.P.D\ resin}$$

3.5. Adsorption studies with Kinnow peel boiled water (KPBW)

3.5.1. Adsorption equilibrium studies:

Known amounts of resin and kinnow peel boiled water containing adsorbate (naringin) were added to a conical flask with stoppers and allowed to achieve equilibrium. After few initial runs, it was observed that it took less than 24 h to reach equilibrium. As there was no loss of naringin, mass balance has been used to determine the amount of naringin adsorbed on the resin as given below. The studies were carried out at temperature 27± 2^{o}C.

$$q_e = \frac{V(C_o - C_e)}{w} \qquad (3.1)$$

where $q_e\,(kg/kg)$ is the amount of naringin adsorbed at equilibrium, $V(m^3)$ is the volume of solution (KPBW), $C_o\,(kg/m^3)$ is the initial concentration of naringin in solution, $C_e\,(kg/m^3)$ is the equilibrium concentration of naringin in solution and $w\ (kg)$ is a weight of the resin.

Experimental procedure:

Equilibrium studies were carried out in conical flasks. Fifty ml of kinnow peel boiled water solution were transferred into 5 different 250 ml conical flasks and numbered as 1, 2, 3, 4, and 5 and 100 ml of kinnow peel boiled water solution were transferred into 3 different 250 ml conical flasks and numbered as 6, 7, and 8. Different known amounts of FPD resin were added to all the 8 conical flasks. Another sample of FPD from the same batch was transferred to an oven to determine solid content. The contents in all 8 conical flasks were shaken for 24 h to reach equilibrium**.** The supernatant solution of each flask was analyzed for naringin content by using Davis test. The same procedure was adopted for fresh, dry, and dropped peels with resins PA-500 and PA-800.

3.5.2. Adsorption kinetic studies:

Batch kinetic studies were carried out in a three-necked round-bottomed 500 ml glass flask fitted with Teflon stirrer. Preliminary investigations showed that there was no effect of stirrer speed on naringin pickup on the resin at 250±10 rpm. Kinnow peel boiled water (450 ml) was transferred to the flask, and the known amount of resin was added to it. The stirrer speed was kept at 250 ± 10 rpm to eliminate mass-transfer as limiting step. Samples of kinnow peel boiled water were drawn at different time intervals to know the variation of naringin concentration in the flask with time. All the experiments were carried out at room temperature 27 ± 2^{o}C. The set up for kinetic studies is shown in Figure 3.6.

Experimental procedure:

A weighed sample of filter paper dried resin was used for kinetics experiments. Another sample from the same batch was transferred to an oven to determine solid content in all the kinetic studies.

Five hundred ml of kinnow peel boiled water solution was taken in a measuring flask, and 50 ml was removed by pipette to get exactly 450 ml solution. The experiment was carried out in a 500 ml three-necked round bottom flask fitted with the stirrer. A known amount of resin was transferred to experimental flasks and stirrer was made on. One (1.0) ml samples were drawn at different times (7.5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 600, and 1440 min). Samples were analyzed for naringin content. The same procedure was adopted for fresh, dry, and dropped peels with resins PA-500 and PA-800.

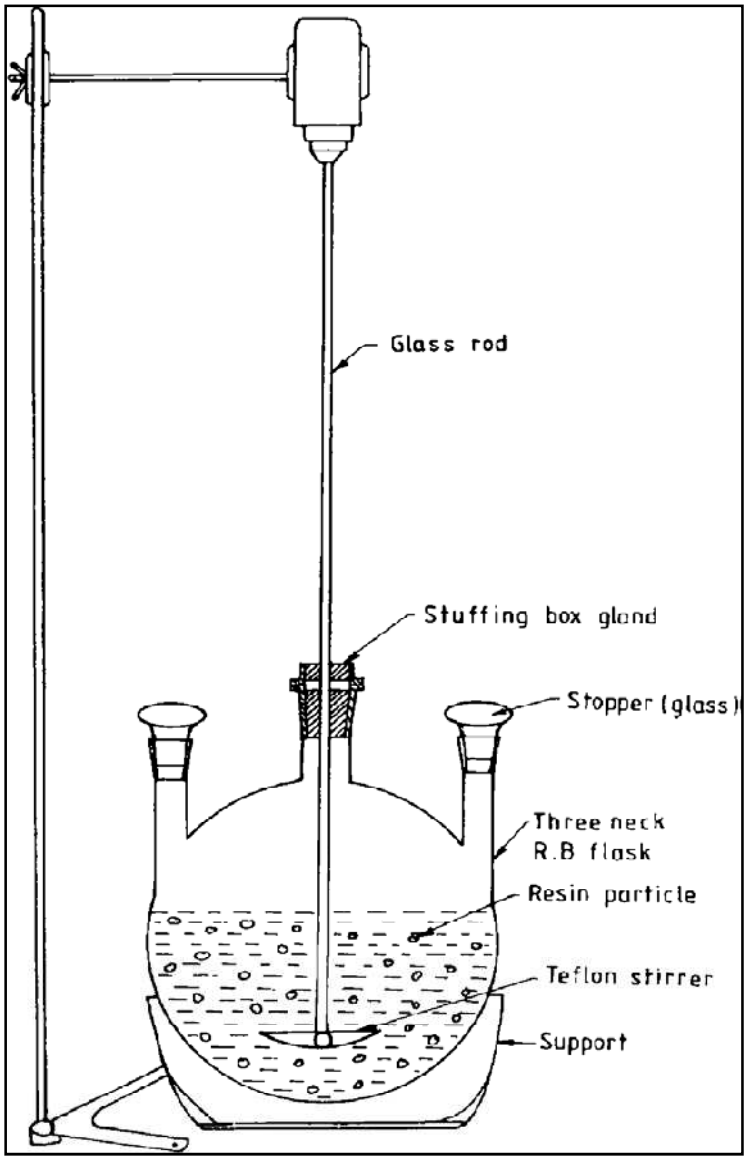

Figure 3.6: Set-up for kinetic studies

3.5.3. Adsorption column studies (Breakthrough curve)

A column studies were carried out in a glass column with ID 14 mm and height about 1.5 m. A glass bulb of 250 ml was fitted above the column by glass blowing; it was used to maintain the level of solution constant. The setup used in this study is shown in Figure 3.7.

To carry out column studies, the glass column was filled with resin up to predetermined height, saturated with citric acid, after draining the citric acid solution. The kinnow peel boiled water was filled up to a fixed height. The flow rate of KPBW remaining after naringin adsorption leaving the column was kept constant at a rate of 2 ml/min (3.3×10^{-8} m^3/s). Samples were collected for 1 min each at intervals of 60 minutes and analyzed for naringin content. The same procedure was adopted for fresh, dry, and dropped peels with the resins PA-500 and PA-800.

3.6. Desorption studies with ethanol

3.6.1. Desorption equilibrium studies

Equilibrium studies were carried out in conical flasks. An amount of 25 ml of ethanol was transferred into 5 different 250 ml conical flasks and numbered as 1, 2, 3, 4, and 5. Fifty ml of ethanol was transferred into 3 separate 250 ml conical flasks and numbered as 6, 7, and 8. Different known amounts of FPD naringin saturated resin was added to the all the 8 conical flasks. The naringin saturated resin was obtained from the adsorption column studies with kinnow peel boiled water. The contents in all 8 conical flasks were shaken for 24 h to reach equilibrium**.**

The amount of naringin desorbed from resin saturated with naringin was calculated using mass balance.

$$W^I(q_{od} - q_{ed}) = V_e C_{ed} \qquad (3.2)$$

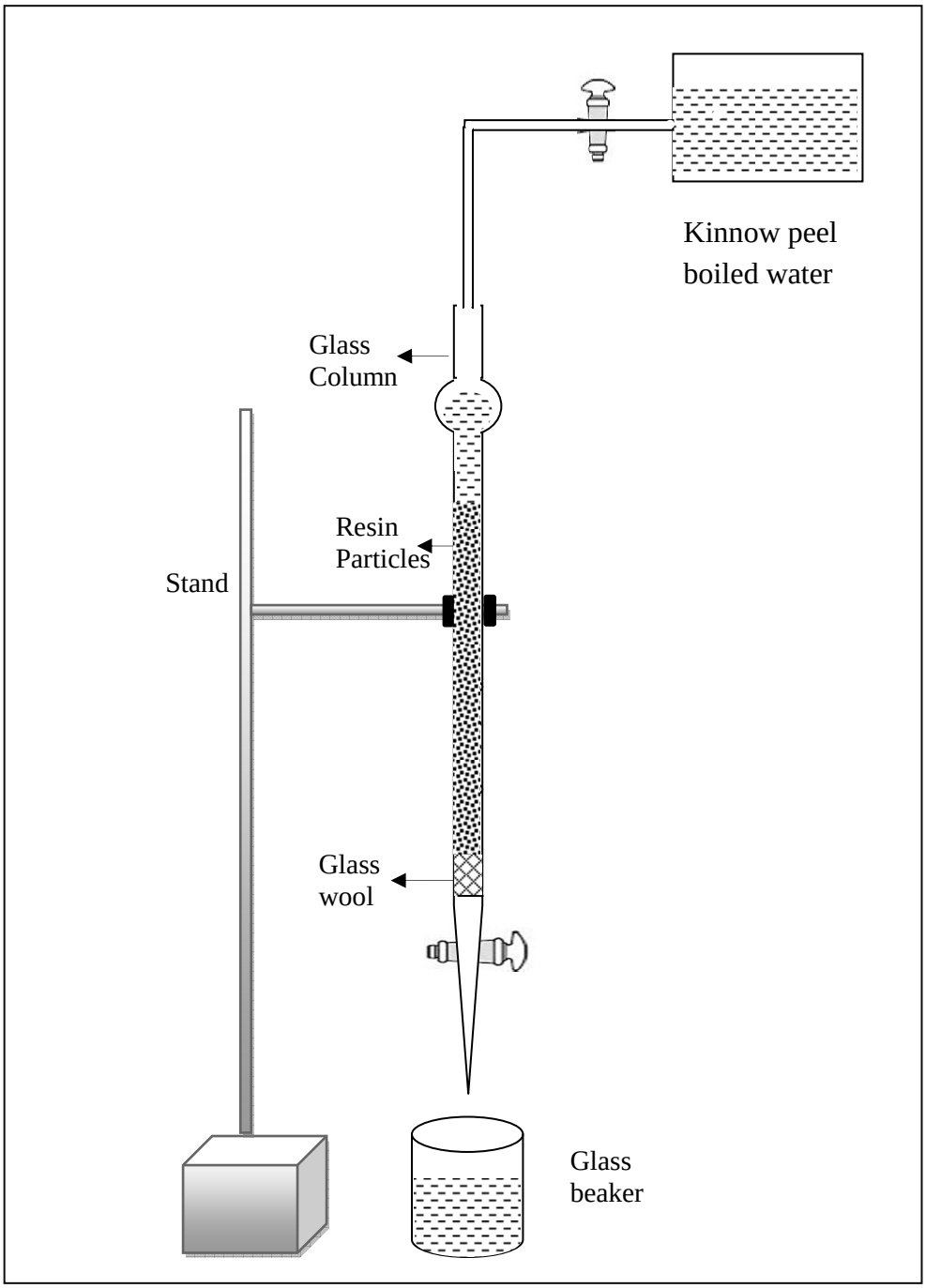

Figure 3.7: Set-up for column studies

where, $W^I(kg) = (w_n + w_f)$ weight of resin saturated with naringin, which was obtained from peel boiled water column studies, $w_n(kg)$ = weight of naringin adsorbed on resin, was obtained from equilibrium data, corresponding to concentration of kinnow peel boiled water used in adsorption column study, $w_f(kg)$ = weight of the resin without naringin, q_{od} = Initial naringin content in the resin, $q_{ed}(kg/kg)$ = amount of naringin desorbed from the resin in ethanol at equilibrium, $V_e(m^3)$ = volume of ethanol, $C_{ed}(kg/m^3)$ = final concentration of naringin at equilibrium in ethanol solution.

The same procedure was adopted for fresh, dry, and dropped peels with resins PA-500 and PA-800.

3.6.2. Desorption kinetic studies

A weighed sample of filter paper dried resin (saturated with naringin which was obtained from adsorption column studies with kinnow peel boiled water) was used for kinetics experiments. Another sample from the same batch was transferred to an oven to determine solid content during kinetic studies. The experiments were conducted in 500 ml three-necked round bottom flask fitted with the stirrer. Five hundred ml of ethanol solution was taken in a measuring flask, and 50 ml was removed by pipette to get exactly 450 ml solution. The filter paper dried resin (saturated with naringin which was obtained from adsorption column studies for kinnow peel boiled water) was transferred to the experimental flask and stirrer was made on. About 2.0 ml of samples was drawn at different times 7.5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 600, and 1440 min. Samples were analyzed for naringin content by following the procedure mentioned in section 3.2. The same procedure was adopted for all the systems.

3.6.3. Desorption column studies

The desorption column studies were carried out in the same setup (Figure 3.5), as used in adsorption column studies. The column was filled with resin saturated with naringin (obtained from adsorption column studies with kinnow peel boiled water). The pure ethanol was filled up to the sufficient height, and the flow rate of the naringin-ethanol solution coming out of column was kept constant at 2 ml/min (3.3×10^{-8} m^3/s). Samples were collected for 1 min at intervals of 60 min. Samples were analyzed for naringin content according to the procedure mentioned in section 3.2. The same procedure was adopted for fresh, dry, and dropped peels with resins PA-500 and PA-800.

3.7. Distillation, filtration, and evaporation of desorbed naringin-ethanol solution

The simple distillation (Figure 3.8) was carried out to concentrate 1.5 litres of the desorbed ethanol-naringin solution to about 150 ml. A Whatman filter paper no. 42 was used to filter the concentrated ethanol-naringin solution. Then the filtrate was passed through a polyester membrane syringe filter (size 0.45 µm) followed by evaporation to recover naringin as a solid.

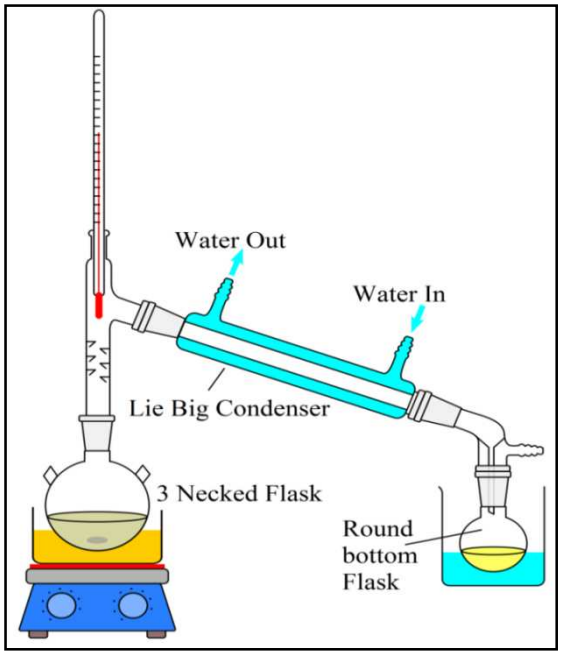

Figure 3.8: Set-up for simple distillation

3.8. Regeneration of resin after ethanol desorption

To know the reusability of the resin after desorption, the resin was regenerated. Adsorption equilibrium studies were carried out to have an idea of the extent to which the resin may be reused for repeated cycles. Initially, the column was filled with ethanol desorbed resin. The warm (about 50^{o}C) sodium hydroxide (NaOH) of morality 1M was filled up to a predetermined height. The flow rate of the liquid stream leaving the column was kept constant at a rate of 2 ml/min (3.3×10^{-8} m^3/s). The sodium hydroxide solution was passed through the bed until the solution leaving the column was colorless. It is an indication that now there is no desorption of naringin, the resin thus obtained was washed with distilled water till complete removal of sodium hydroxide (NaOH). This resin is referred as regenerated resin.

Studies with regenerated resins:

All adsorption-desorption equilibrium, kinetic, and fixed bed column studies with regenerated resins were carried out as per the same procedures described in the sections 3.5 and 3.6.

3.9. Recovery of Naringin

Recovery of naringin from the column (on the basis of naringin present in KPBW) was calculated by using the formula given below.

Recovery of naringin from KPBW

$$= \frac{Wt.\ of\ \ obtained\ \ naringin \times purity}{liters\ of\ \ KPBW\ \ \ passed \times conc.\ of\ \ naringin\ in\ KPBW}$$

The purity of naringin sample was determined as follows: 0.5 g of the sample was dissolved in 1 litre of distilled water. The concentration of naringin in solution was determined by Davis test and purity was determined as

$$= \frac{Concentration\ of\ \ solution\ in\ \ ppm}{500}$$

3.10. Recovery of Pectin and characterization

Recovery of pectin (basis on pectin present in KPBW) was calculated by using the formula given below.

$$\text{Recovery of crude pectin} = \frac{Wt.\ of\ \ crude\ \ pectin \times conc.\ of\ obtained\ \ pectin\ as\ \ AuA}{liters\ of\ \ KPBW\ \ passed \times conc.\ of\ \ pectin\ in\ KPBW}$$

Characterization of Pectin

The obtained pectin was characterized by estimation of parameters Moisture content, Ash content, Equivalent weight, Methoxyl content, Anhydrounic acid, and Degree of esterification (DE). These parameters were determined by the following procedures.

Moisture content determination (Ranganna, 2005): An empty petri dish was dried in an oven, cooled in a desiccator and weighed. One g of the pectin sample was transferred into the crucibles in the oven and heated at 130°C for 1 h. The petri dish was cooled to room temperature in a desiccator and weighed.

$$\text{Moisture content (\%)} = \frac{Wt.\ of\ \ pectin\ sample\ after\ \ drying}{Wt.\ of\ \ pectin\ sample} \times 100$$

Ash content determination (Ranganna, 2005): About 1 g of the pectin sample was accurately weighed into a weighed empty crucible separately. The crucible was placed in a furnace and heated for 3-4 h at 600°C to burn off all the organic matter. The crucible was taken out of the furnace and placed in a desiccator to cool and weighed.

$$\text{Ash content (\%)} = \frac{Wt.\ of\ \ ash}{Wt.\ of\ \ sample} \times 100$$

Equivalent weight determination (Ranganna, 2005): Weighed 0.5 g pectin sample and transferred into a 250 ml conical flask and 5 ml ethanol, 1.0 g sodium chloride were added.

One hundred ml of distilled water and few drops of phenol red indicator were added to the mixture. Care was taken to ensure that all the pectin had dissolved and that no clumping occurred at the sides of the flask. After that, the solution was slowly titrated (to avoid possible de-esterification) with 0.1 N NaOH to endpoint (pink color).

$$\text{Equivalent weight} = \frac{Wt.\ of\ \ pectin\ sample}{ml\ of\ \ alkali \times Normality\ of\ \ alkali} \times 1000$$

Methoxyl content (MC) determination (Ranganna, 2005): To the neutral solution titrated for equivalent weight, containing 0.5g pectic substances, 25 ml of 0.25 N NaOH was added and shaken thoroughly. It was allowed to stand for 30 min at room temperature in a stoppered flask; after that 0.25 N HCl was added. The contents were titrated with 0.1 N NaOH until the end point reached (pink color).

$$\text{Methoxyl content (\%)} = \frac{ml\ of\ \ alkali \times Normality\ of\ \ alkali \times 3.1}{Wt.\ of\ \ sample}$$

Anhydrounic acid(Ranganna, 2005): To determine the alkalinity of ash, the ash was dissolved in 25 ml of 0.1 N HCl. The contents were heated and cooled to a room temperature. This mixture was titrated with 0.1 N NaOH using phenolphthalein indicator until the end point was reached (orange color).

$$\text{Alkalinity \% as carbonate} = \frac{Volume\ of\ \ NaOH \times Normality\ of\ \ NaOH \times 60 \times 100}{Wt.\ of\ \ ash \times 1000}$$

Anhydrounic acid

$$= \frac{176(m.e\ alakli\ \ for\ \ free\ acid \times m.e\ alakli\ \ for\ \ soponification \times m.e\ titrable\ ash)}{Wt.\ of\ \ sample\ (mg)} \times 100$$

where m.e is milliequivalent, Alkali for free acid is obtained from ash alkalinity of ash, Alkali for soponification is obtained from equivalent weight determination, and Titrable ash is obtained from ash content determination

Degree of esterification (DE) (Ranganna, 2005): The DE of pectin was measured on the basis of methoxyl and AuA content and calculated by using the formula.

$$\text{Degree of esterification (\%)} = \frac{176 \times \%\textit{methoxyl content}}{31 \times \%\textit{anhydrounic acid}} \times 100$$

Sources of errors in experimental observations

1. The concentration of naringin and pectin was determined from calibration chart. The millimetre part on the graph was read with a least count of 5 ppm. Further, on spectrophotometer absorbance could be detected to an accuracy of ±0.001. Thus an error of 10ppm may occur in any measurement.

2. The pipette of 0.1 ml with least count of 0.001 ml was used for pipetting naringin solution. During pipetting an error may occur: the solution may remain inside the pipette.

3. In kinetic studies, the samples were drawn at different times from agitating mixture. It takes 25-45 (s) in withdrawing the sample from an agitating mixture solution. During early stages of a kinetic experiment change in solution, concentration is very sharp (Particularly when adsorbent loading is high). Therefore it was very tough to withdraw samples at predetermined time intervals during the initial stages.

4. In column study with the solution, the major problem was to maintain a constant flow rate of the outgoing solution.