CHAPTER 4

CONVERSION OF HYDROXY CITRIC ACID INTO STABLE SALTS

4.1 INTRODUCTION

As described earlier, hydroxy citric acid (HCA) is an alpha, beta di-hydroxy tri-carboxylic acid. Since the carbon atom at position-2 is chiral (Fig.1.2), it easily lactonises to the corresponding lactone [Lewis and Neelakantan 1965]. Depending on the position of the hydroxyl groups two types of lactones are formed [Stallings *et al.* 1979] (Fig. 1.4).

These lactones are easily hydrolysable using strong alkalies or strong acids. The free hydroxy citric acid liberated is converted into different salts. Here in this chapter, the formation of lactones and preparation of calcium hydroxy citrate, potassium hydroxy citrate, magnesium hydroxy citrate and sodium hydroxy citrate are described. Preparation of different grades of calcium hydroxy citrate are also explained.

Depending on the cation, the hydroxy citrates formed have different properties. These properties characterize the end use of these salts.

The physical and chemical properties of these hydroxy citrates are also explained in this chapter.

4.2 MATERIALS

Dried rinds of *Garcinia cambogia* fruit grown in Angamaly were used for the studies. The chemicals used are of AR grade.

4.3 METHODS

4.3.1 Preparation of water extract from Garcinia cambogia rind.

Water extract was prepared from *Garcinia cambogia* rind as described in Chapter 2. HCA content was estimated and this extract was used for further studies.

4.3.2 Preparation of Hydroxy citric acid Lactone

Hydroxy citric acid lactone was prepared according to the procedure explained in Chapter 2.

4.3.3 Preparation of Calcium hydroxy citrate

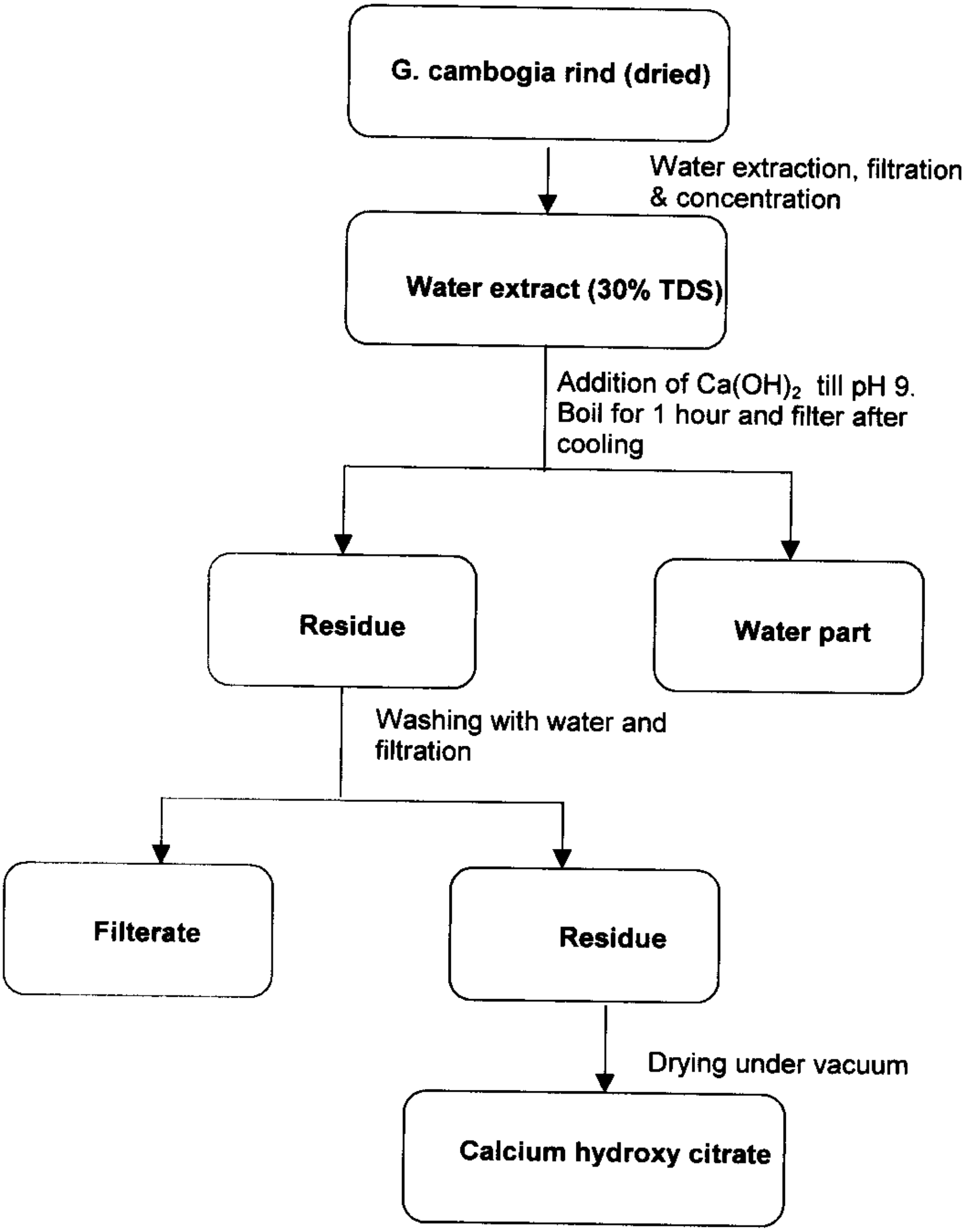
HCA present in the water extract was converted into calcium hydroxy citrate by reacting with calcium hydroxide. Water extract was prepared as described in 2.2.1. The total dissolved solid of the extract was noted. To the crude water extract taken in a 1000mL beaker, calcium hydroxide solution was added drop by drop until the pH became 9. Calcium hydroxy citrate precipitated at the bottom was collected by filtering under vacuum. This residue was washed with water to remove excess calcium hydroxide, if any. The washed material was dried in an air oven at 110°C for about 4–6 hours till the powder was free of moisture. This final product obtained was calcium hydroxy citrate. The yield of calcium hydroxy citrate mainly depends on TDS of initial water extract and pH of final neutralized solution. Water extract with different TDS level were used for this study. In another study, different pH were maintained during neutralization to know yield variation of calcium hydroxy citrate. The detailed flow diagram was shown in Figure 4.1.

4.3.4 Preparation of high Bulk Density Calcium hydroxy citrate

In pharmaceutical or neutraceutical industry, the bulk density of ingredients determines filled weight per capsule. Normally this calcium hydroxy citrate has bulk density around 0.35 – 0.55g/mL. We had done some work to increase bulk density to 0.7 – 0.9g/mL.

Calcium hydroxy citrate is a light brownish free flowing powder, which was passed through a Roll Compactor with 200mm diameter, 50mm width at different RPM of Roll and Feed screw.

Fig. 4.1: Flow diagram for the preparation of Calcium hydroxy citrate.



4.3.5 Preparation of compressible granules of Calcium hydroxy citrate

Compressible granules are used for making tablets. Hence we have done some works to find out the optimum conditions to get good quality compressible granules of calcium hydroxy citrate. The conditions include the quantity of gum and drying level required during process.

The gum used was CMC (Carboxy methyl cellulose), which was dissolved in water (3% w/w) along with methyl paraben (0.05%) as a preservative. The calcium hydroxy citrate was added slowly into the solution, the resulting mixture had 35% water. The resulting mixture was kept for half drying and moisture level reduced to 15%. This half dried material was passed through the granulator and then sieved according to mesh. Final drying was done to attain moisture level below 4%.

This final product is mainly used for manufacturing tablets and often known as direct compressible form. Here the optimum conditions like moisture content, amount of gum and methyl paraben were found out.

4.3.6 Preparation of Potassium hydroxy citrate

As per the procedure described in Chapter 2, potassium salt of HCA was prepared by reacting potassium hydroxide with water extract of *Garcina cambogia* and subsequent settling with methanol. Potassium hydroxy citrate obtained was insoluble in methanol, but clearly soluble in water.

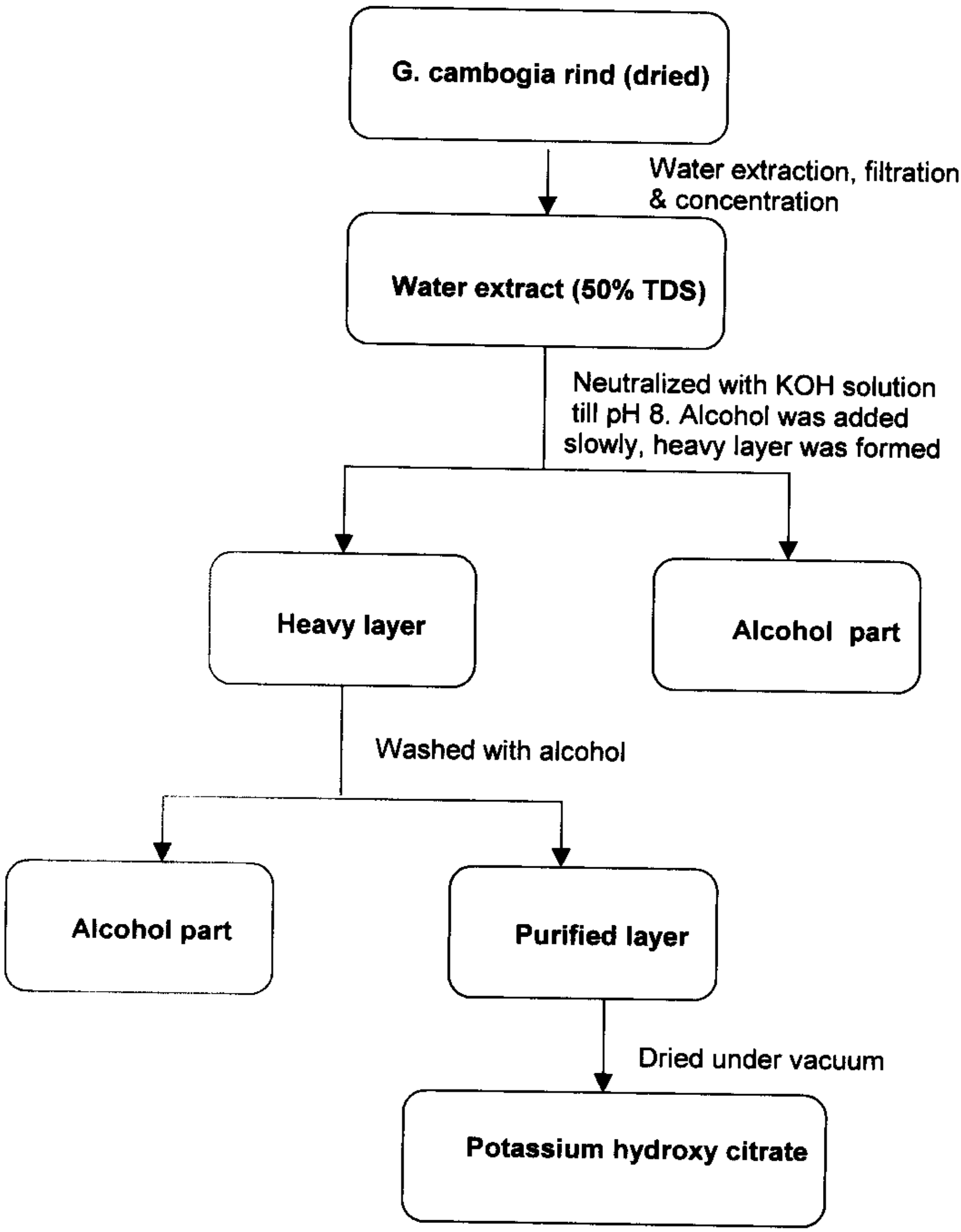
The yield of potassium hydroxy citrate depends on two factors.

1. Total dissolved solids of neutralized solution
2. Amount of alcohol added for settling

Water extract was made as per procedure explained in Chapter 2.

20% potassium hydroxide solution was added into the water extract taken in a beaker with constant stirring. Addition of alkali, continued till the pH of reacting solution reached upto 8 for minimum 5 minutes. The whole neutralized solution was concentrated for getting different TDS level. Different quantity of alcohol were used for settling the heavy layer. This heavy layer was taken out and dried under vacuum. The dried material obtained was potassium hydroxy citrate. The detailed flow diagram is given in Figure 4.2.

Fig. 4.2: Flow diagram for the preparation of Potassium hydroxy Citrate



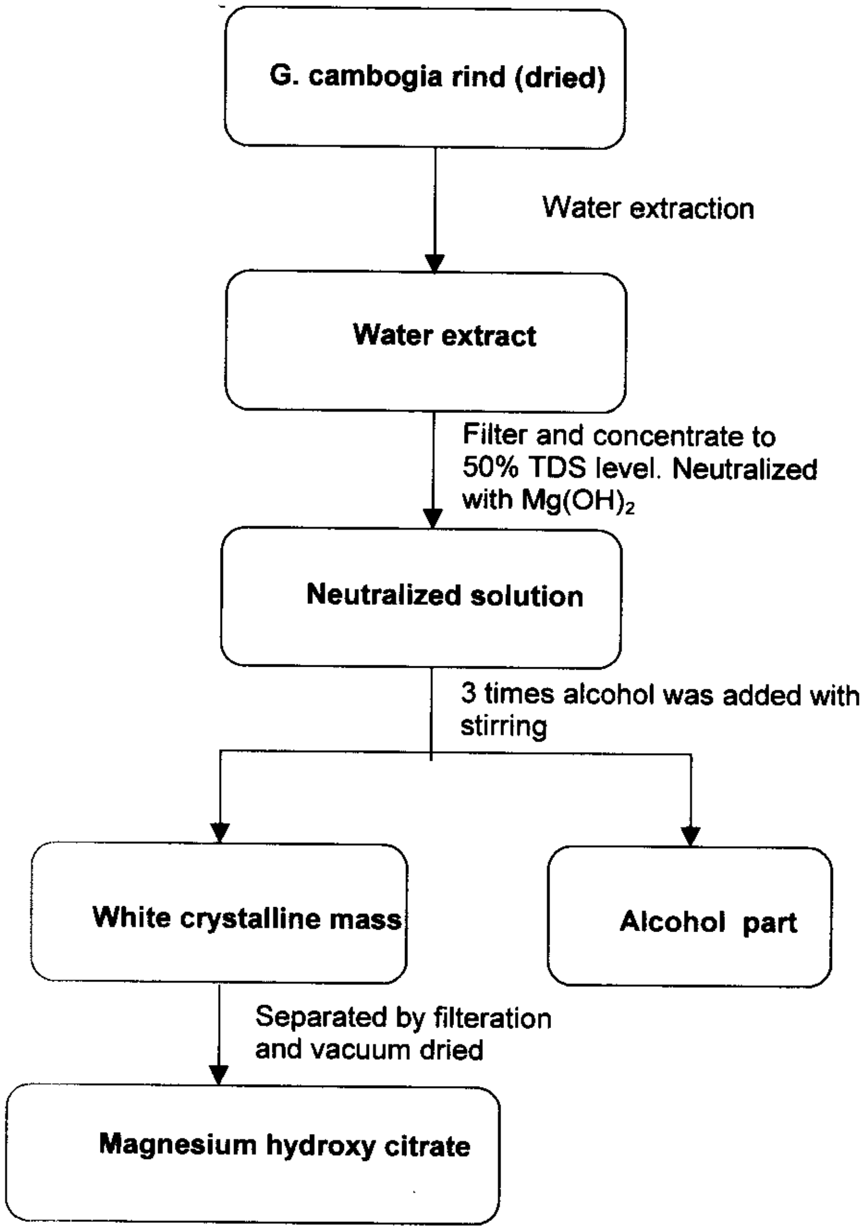
4.3.7 Preparation of Magnesium hydroxy citrate

Magnesium hydroxy citrate was made by reacting magnesium hydroxide and water extract of *Garcinia cambogia* rind and precipitating by alcohol.

Water extract of *Garcinia cambogia* rind was prepared as explained in chapter 2. Water extract was filtered through Whatman No.1 filter paper under vacuum. Fifty percent (50%) magnesium hydroxide solution was added slowly, while stirring and warming. The reaction was allowed to continue till the pH attained 7.5, stirring and heating continued for 30 minutes.

This neutralized solution was cooled to room temperature, to which three times alcohol (by volume) was added slowly with stirring. A creamy white precipitate was formed, which was filtered and washed again with alcohol and finally dried under vacuum. This dried material represents magnesium hydroxy citrate. The detailed flow diagram is shown in Figure 4.3.

Fig. 4.3: Flow diagram for the preparation of Magnesium hydroxy citrate



4.3.8 Preparation of Sodium hydroxy citrate

Preparation of sodium hydroxy citrate is very similar to that of potassium hydroxy citrate, but sodium hydroxide was used instead of potassium hydroxide. The optimum conditions such as total dissolved solids of reacting solution and volume of alcohol were same as that of potassium hydroxy citrate. The flow chart for the preparation of sodium hydroxy citrate is shown in Figure 4.4.

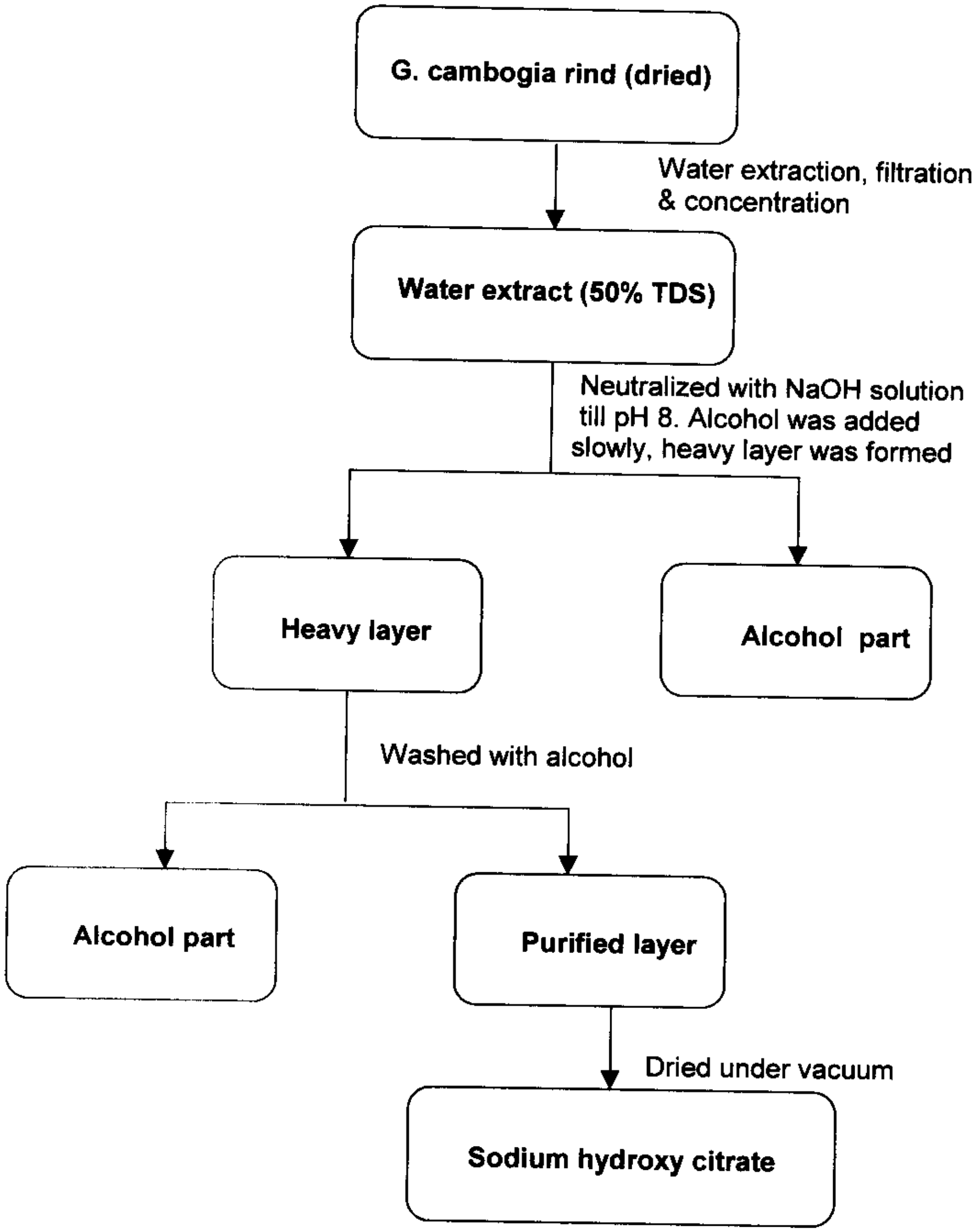
4.3.9 Checking the solubility

Solubility of the salts prepared was checked by adopting the procedure explained in Chapter 2. Solubility of the hydroxy citrates in water and dilute hydrochloric acid were noted.

4.3.10 Checking the pH

The pH of the salt solutions prepared was noted by using the procedure described in Materials and Methods.

Fig. 4.4: Flow diagram for the preparation of Sodium hydroxy citrate



4.3.11 Checking the Bulk density

Bulk density (BD) is an important parameter for pharmaceutical grade powders because BD determines the filled weight of capsules. The BD of the prepared samples was checked as described in Chapter 2.

4.3.12 Determination of Moisture

Moisture content of the hydroxy citrate salts was determined by Toluene method as explained in Chapter 2.

4.3.13 Estimation of HCA by UV spectrophotometric method

Hydroxy citric acid content of the prepared samples were estimated by UV–method as developed by Benny Antony *et al.* (1999) which is described in Chapter 5.

4.3.14 Checking the Salt content

Salt content of the hydroxy citrates prepared were checked by Mohr's method, which is described in Materials and Methods.

4.3.15 Estimation of Citric acid

Citric acid content was estimated by Pentabromoacetone method, which is explained in Chapter 2

4.3.16 Estimation of Calcium

Calcium content of calcium hydroxy citrate was estimated by Permanganometric method as shown in Materials and Methods.

4.3.17 Estimation of Potassium

Potassium content of potassium hydroxy citrate was estimated as described in Chapter 2.

4.3.18 Estimation of Magnesium

Magnesium content of magnesium hydroxy citrate was found out by using the procedure described in Chapter 2.

4.3.19 Estimation of Sodium

Percentage of sodium in the sodium hydroxy citrate was determined using the procedure described in Materials and Methods.

4.3.20 Analysis of Heavy metals, Aflatoxin, Crop contaminants and Microbiological parameters

Presence of Heavy metals, Aflatoxin, Crop contaminants, like saffrole, hydrocyanic acid, agaric acid and Microbial parameters like Total plate count, Yeast and mould, E-coli, Salmonella and Staphylococcus were analysed at outside laboratories as indicated in Chapter 2.

4.4 RESULTS

4.4.1 Preparation and properties of Hydroxy citric acid Lactone

HCA lactones were prepared from water extract of *Garcinia cambogia* and the properties were studied. The results obtained are shown in Table 4.1. The physical and chemical analysis of lactones confirmed its identity.

Table 4.1: Physical and chemical properties of Hydroxy citric acid Lactone

Sl. No.	Property	HCA lactone *
1	Melting point	177°C
2	Optical rotation (2% solution)	+ 101°
3	Crystal shape	Needles
4	Solubility	Very soluble in alcohol and water and fairly soluble in ether
5	Paper chromatography N – Butanol : formic acid : water 4:1:5 (Whatmann No. 2 paper)	Rf -0.44
	N – Propanol – ammonia – water 4:1:5 (Whtaman No. 2 paper)	Rf -0.25
	Meta vanadate spray	Yellow colour

* The results are the average of three experiments

4.4.2 Preparation of Calcium hydroxy citrate

Among the different salts of Hydroxy citric acid, calcium hydroxy citrate is the most widely used form, especially in the tablets and capsules. While manufacturing this product the calcium hydroxide breaks the lactones, forming insoluble calcium salt of hydroxy citric acid. The yield of this salt depends on pH of neutralized solution. We have studied the formation of calcium hydroxy citrate at different pH range, from 7–10 during neutralization. The optimum pH at which maximum yield of calcium hydroxy citrate formed was 9-10. At lower pH the yield was low and the lactone content in the final product was high. The data is shown in the Table 4.2.

Table 4.2: Yield of calcium hydroxy citrate at different pH during neutralization

Quantity of water extract of rind*	pH of water extract after adding $Ca(OH)_2$	Yield of cal. hydroxy citrate**	% of lactone in the final product**	% of HCA in the final product**
100g	7	15g	5.2	49.2
100g	8	18g	4.5	50.5
100g	9	21g	3.2	54.6
100g	10	22g	2.9	55.1

*** Water extract with 30% TDS**

**** All results are expressed on dry weight basis and these values are the average of 3 – 4 experiments**

TDS level of water extract is also a yield determining factor. In order to find out the effect of TDS on the yield of calcium hydroxy citrate water extract with different TDS had been reacted with $Ca(OH)_2$. The results obtained are shown in the Table 4.3.

Table 4.3: Effect of TDS level of water extract on the yield of calcium hydroxy citrate

TDS of water extract of Garcinia cambogia fruit	pH	*Yield of Calcium Hydroxy citrate
10%	9	45%
30%	9	69%
50%	9	55%
70%	9	48%

* Yield means % of dry calcium salt with respect to TDS of solution

The maximum yield of calcium salt was obtained with water extract having 30% Total dissolved solids. With higher TDS level, yields were low because of high concentration of acid with less quantity of water. All reactions were done at pH 9.

At low TDS level reaction was completed but some quantity of calcium salt was washed out through the filtrate during filtration, resulting a low yield

4.4.3 Physical and chemical properties of Calcium hydroxy citrate

The calcium hydroxy citrate prepared was analyzed for physical and chemical parameters, which are summarized in the Table 4.4. Clear solubility in dilute HCl provided the information on the purity of salt.

4.4.4 Preparation of calcium hydroxy citrate having high Bulk density

In pharmaceutical or neutraceutical industry, the bulk density of ingredients, determine the filled weight per capsule. Normally this calcium hydroxy citrate has bulk density around 0.35 – 0.55g/cc. Here we have done some work to increase bulk density to 0.7 - 0.9g/cc.

The optimum conditions to make high-density calcium hydroxy citrate were studied. Roll Compactor with 200 mm diameter, 50 mm width were used for this study. The maximum bulk density was obtained when RPM of Roll and feed screw kept at 15 and 42 respectively. The other salts of HCA didn't work with Roll Compactor, because of their hygroscopic character.

Analysis of this material is shown in Table 4.5

Table 4.4 : Physical and chemical properties of Calcium hydroxy citrate

Sl. No.	Properties	Calcium hydroxy citrate
1	Colour and appearance	Light tan, free flowing powder
2	Odour	Characteristic, smokey herbal
3	Solubility in water	75%
4	Solubility in dilute HCl	99.2%
5	pH (1% solution)	8.0
6	Bulk density	0.51 g/mL
7	Moisture content	4.3%
8	HCA (Spectrophotometric method)	53.1%
9	Salt content (Mohr's method)	4.3%
10	Citric acid	3.1%
11	Lactones	1.2%
12	Calcium content	18.9%

Table 4.5 : Bulk densities of Calcium hydroxy citrate

Sl . No.	Bulk density of normal product *	Bulk density of new product *	Roll RPM	Feed screws RPM
1	0.51g/mL	0.91g/mL	15	42
2	0.53g/mL	0.95g/mL	15	42
3	0.50g/mL	0.92g/mL	15	42
4	0.55g/mL	0.98g/mL	15	42

* The results are average of two analyses

4.4.5 Preparation of compressible granules of Calcium hydroxy citrate

Compressible granules are used for making tablets. We have done some experiments to find out the optimum conditions to get good quality compressible granules of calcium hydroxy citrate. These conditions include the quantity of gum used and drying level during processing.

The gum used was CMC (Carboxy methyl cellulose), which was dissolved in water (3% (w/w)) along with methyl paraben (0.05% (w/w)) as a preservative. The calcium hydroxy citrate was added slowly into the above solution. The resulting mixture having 35% water was kept for half drying and moisture level reduced to 15%. This half dried material was passed through the granulator and then sieved according to mesh. Final drying was done to attain moisture level below 4%.

This final product is mainly used in manufacturing tablets and often known as direct compressible form. Here the optimum conditions like level of water used, amount of gum and methyl paraben added were found out, to produce good quality direct compressible granules of calcium hydroxy citrate.

4.4.6 Preparation of Potassium hydroxy citrate.

As described in the preparation of potassium hydroxy citrate, the yield of potassium hydroxy citrate depends on the Total dissolved solids of neutralized solution. Neutralized solution with different Total dissolved solids ranging from 30 – 90% were used for this study with the same quantity of alcohol. The data is shown in the Table 4.6.

Table. 4.6 : Effect of TDS of neutralized solution on the yield of potassium hydroxy citrate

TDS of neutralized solution (100mL)	Amount of alcohol added (300mL)	Yield of potassium hydroxy citrate*	HCA percentage#
30%	3 times	20.2%	51%
50%	3 times	40.5%	52.5%
70%	3 times	50.1%	52%
80%	3 times	45.7%	51.8%
90%	3 times	44.4%	52%

*** Yield means percentage of potassium hydroxy citrate obtained with respect to Total dissolved solids. The experiment is conducted thrice and average value was taken**

 # Percentage of HCA was analysed twice and average was noted

The maximum yield of potassium hydroxy citrate was obtained from a neutralized solution with 70% TDS. Alcohol level was kept constant during all experiments.

Amount of alcohol used for settling has an influence on the yield of potassium hydroxy citrate. Different amounts of alcohol were used for the study. The result obtained is shown in Table. 4.7.

Table 4.7: Effect of volume of alcohol on the yield of Potassium hydroxy citrate.

Vol. of neutralized solution (TDS is 70%)	Amt. of alcohol used	Weight of potassium hydroxy citrate obtained (g)	HCA percentage*
50mL	50mL	10	49.5%
50mL	100mL	12	50.4%
50mL	150mL	17.3	51.6%
50mL	200mL	17.9	52.1%

* Percentage of HCA was analysed twice and average was noted

The yield of potassium hydroxy citrate was maximum when 3-4 times alcohol was used. This finding is very important for commercial production of potassium hydroxy citrate. The yield of potassium hydroxy citrate can be increased by optimising the volume of alcohol during production.

4.4.7 Physical and chemical properties of Potassium hydroxy citrate

The potassium hydroxy citrate prepared was analysed for physical and chemical properties and the results obtained are summarized in Table 4.8.

Table 4.8 : Physical and chemical properties of Potassium hydroxy Citrate

Sl. No.	Properties	Potassium hydroxy citrate
1	Colour and appearance	Light brownish crystalline hygroscopic powder
2	Odour	Characteristic, smokey
3	Solubility in water	Clearly soluble
4	Solubility in dilute HCl	Clearly soluble
5	pH (1% solution)	8.5
6	Bulk density	0.48 g/mL
7	Moisture content	3.9%
8	HCA (Spectrophotometric method)	50.9%
9	Salt content (Mohr's method)	4.5%
10	Citric acid	3.9%
11	Lactones	1.8%
12	Potassium content	15.1%

4.4.8 Preparation of Magnesium hydroxy citrate

Preparation of magnesium hydroxy citrate is closely similar to that of potassium hydroxy citrate. Instead of potassium hydroxide, magnesium hydroxide was used for neutralizing the water extract. The optimum conditions of Total dissolved solids levels; pH and volume of alcohol were studied for the preparation of potassium hydroxy citrate and same as that of magnesium hydroxy citrate. The chemical and physical properties of magnesium hydroxy citrate are shown in the Table 4.9.

HCA, bulk density and salt content of magnesium hydroxy citrate were higher than that of potassium hydroxy citrate.

Table 4.9 : Physical and chemical properties of Magnesium hydroxy citrate

Sl. No.	Properties	Magnesium hydroxy citrate
1	Colour and appearance	Off white crystalline free flowing powder
2	Odour	Characteristic
3	Solubility in water	97.8% soluble
4	Solubility in dilute HCl	Clearly soluble
5	pH (1% solution)	8
6	Bulk density	0.63 g/mL
7	Moisture content	4.1%
8	HCA (Spectrophotometric method)	55.5%
9	Salt content (Mohr's method)	6.4%
10	Citric acid	2.2%
11	Lactones	1.9%
12	Magnesium content	10.9%

4.4.9 Preparation of Sodium hydroxy citrate

The procedure employed for the preparation of sodium hydroxy citrate was exactly similar to that used for potassium hydroxy citrate and magnesium hydroxy citrate. Instead of potassium hydroxide and magnesium hydroxide, sodium hydroxide was used for neutralization. The optimum conditions such as 70% Total dissolved solids level, 9-10 pH range and 3-4 times volume of alcohol etc. were used for the preparation. The sodium hydroxy citrate formed was dried and analyzed for the physical and chemical properties. The values obtained are given in the Table 4.10.

4.4.10 Comparative study of different Hydroxy citrates

The different salts of hydroxy citrate prepared such as calcium hydroxy citrate, potassium hydroxy citrate, magnesium hydroxy citrate and sodium hydroxy citrate were compared for all quality parameters . The comparative results are summarized in Table 4.11.

Table 4.10 : Physical and chemical properties of Sodium hydroxy citrate

Sl. No.	Properties	Sodium hydroxy citrate
1	Colour and appearance	Off white crystalline powder
2	Odour	Characteristic
3	Solubility in water	Clearly soluble
4	Solubility in dilute HCl	Clearly soluble
5	pH (1% solution)	8
6	Bulk density	0.6 g/mL
7	Moisture content	4.2%
8	HCA (Spectrophotometric method)	52.3%
9	Salt content (Mohr's method)	4.9%
10	Citric acid	3.5%
11	Lactones	1.5%
12	Sodium content	14.3%

Table 4.11 : Physical and chemical properties of Calcium, Potassium, Sodium and Magnesium hydroxy citrates: - A comparative data

Sl. No.	Properties	Hydroxy citrates of			
		Calcium	Potassium	Sodium	Magnesium
1	Colour and appearance	Light tan free flowing powder	Light brown crystalline hygroscopic powder	Off white crystalline powder	Off white crystalline free flowing powder
2	Solubility in water	75% soluble	Clearly soluble	Clearly soluble	Clearly soluble
3	Bulk density	0.51g/mL	0.48g/mL	0.60g/mL	0.63g/mL
4	HCA (Spectrophotometric method)	53.1%	50.9%	52.3%	55.5%
5	Lactones	1.2%	1.8%	1.5%	1.9%
6	Total salt content	4.3%	4.5%	4.9%	6.4%
7	Corresponding acid radical. Calcium/ Potassium/sodium/ magnesium	18.9%	15.1%	14.3%	10.9%

Comparing the colour of the salts, the magnesium and sodium hydroxy citrates are off white colored where as calcium hydroxy citrate is light tan and potassium hydroxy citrate is light brown. Regarding the appearance of the salts, calcium hydroxy citrate is a free flowing amorphous powder whereas all other salts are crystalline powders. Potassium hydroxy citrate and Sodium hydroxy citrate are highly hygroscopic. Magnesium hydroxy citrate is more free flowing than Potassium and Sodium hydroxy citrates, and had least level of mineral content. Calcium hydroxy citrate mainly used in capsules and dry preparations. But magnesium, potassium and sodium salts are being used in beverage type application. The percentage of lactones, in all samples were almost same.

4.4.11 Estimation of Heavy metals, Aflatoxin, Crop contaminants and Microbiological parameters

Presence of heavy metals like Copper, Zinc, Mercury, Arsenic, Cobalt, and Cadmium were analysed for G. cambogia rind and calcium hydroxy citrate. Presence of crop contaminants like Saffrole, Hydrocyanic acid and Agaric acid were measured. Aflatoxin levels in G. cambogia rind and leaves were also noted. Microbial parameters like Total plate count, E-coli, Yeast and mould, Salmonella and Staphylococcus were estimated. The results obtained are also shown in Table 4.12.

Table 4.12 Estimation of heavy metals, Aflatoxin, Crop contaminants and Microbiological parameters in G. cambogia rind and Calcium hydroxy citrate

Sl. no	Parameters	Garcinia rind	Calcium hydroxy citrate
1	**Heavy metals**		
	Lead	1 ppm	3 ppm
	Zinc	3 ppm	12 ppm
	Copper	1 ppm	4 ppm
	Arsenic	Not detected	Not detected
	Cadmium	Not detected	Not detected
	Cobalt	Not detected	1 ppm
	Mercury	Not detected	Not detected
2.	Aflatoxin	Not detected	Not detected
3.	**Crop contaminets**		
	Saffrole	5 ppm	Not detected
	Agaric acid	18 ppm	Not detected
	Hydro cyanic acid	2 ppm	Not detected
4.	**Microbial parameters**		
	Total plate count	400-600cfu/g	90cfu/g
	Yeast and mould	10-20cfu/g	< 10cfu/g
	E-coli	Not detected	Not detected
	Salmonella	Not detected	Not detected
	Staphylococcus	Not detected	Not detected

Contents of heavy metals were present in calcium hydroxy citrate more than that in rind. Reason may be the high concentration of extract and contaminations from the reacting vessels. But microbial contamination was less in calcium hydroxy citrate because of the sterilization by heating the final product.

4.5 DISCUSSION

Hydroxy citric acid is the major constituent of *Garcinia cambogia*, an exotic fruit grown in the southern part of India. HCA is regarded recently as a best natural medicine for controlling obesity. It is the major ingredient in the popular dietary supplements marketing for weight reduction and those products are available as OTC drugs. Being an unstable compound, hydroxy citric acid gets easily lactonised and these lactones are biologically inactive. Hence the revolutionary weight reducing product *Garcinia cambogia* extract is being marketed as a salt of Hydroxy citric acid as a stable form of HCA. *Garcinia cambogia* extracts available in the market were standardized with minimum 50% HCA.

Lactones were formed when the solution of HCA was heated and pure lactones were recovered by ion exchange chromatography. Physical and chemical properties confirmed that lactones are different from pure acids.

Lactones were converted back to salts by reacting with alkali. This principle was employed for the production of different salts of HCA, which is found to be most stable form of HCA.

In this study we have found the optimum conditions such as Total dissolved solids of initial water extract and pH during neutralization to convert HCA into different salts with minimum purity of 50% HCA. The physical and chemical properties also were studied.

The total dissolved solids of the solution and pH of the reacting solution are important for the correct formation of calcium hydroxy citrate. It was found that pH at 9 gave the maximum yield of Calcium hydroxy citrate with less level of lactones. Total dissolved solids of initial water extract at 30% are the optimum for maximum yield of calcium hydroxy citrate. The calcium salt formed was easily recovered from the reaction vessel, since it was less soluble in water.

Calcium hydroxy citrate was converted into high-density material using Roll Compactor. The maximum bulk density was obtained when RPM of Roll and screw feeder kept at 15 and 42 respectively. This high bulk density material can be used in capsules for high medication per capsule.

The optimum conditions such as level of gum and moisture were arrived to get compressible form of *Garcinia cambogia* calcium salt, which has a wider application in tablet manufacturing.

Potassium, sodium and magnesium hydroxy citrates are hygroscopic in nature and recovered by alcohol precipitation. The maximum yield of potassium hydroxy citrate were obtained when the Total dissolved solids of neutralized solution were kept at 70% with 3 times by volume of alcohol. The same optimum parameters were noticed in the case of Magnesium and Sodium hydroxy citrate preparation.

The heavy metal analysis showed that more heavy metals present in the calcium hydroxy citrate than in G. cambogia rind. Aflatoxin was not detected in calcium hydroxy citrate, but detected in G. cambogia rind. Microbiological analysis shows that less level in calcium hydroxy citrate but more in rinds. Usually calcium hydroxy citrate is sterilized by heating in the final stage, which lowers microbial contamination.