WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 4, 1555-1572. Research Article ISSN 2277– 7105

IN-HOUSE PROCESSING OF NANO CALCIUM TABLETS

***Divya O., Prakash P., Dedeepya D., Uma P. and Sameer P.M.D.**

Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tirupati, Andhra Pradesh.

Article Received on
20 Feb. 2021,

Revised on 10 March 2021,
Accepted on 30 March 2021

DOI: 10.20959/wjpr20214-20180

***Corresponding Author**
Divya O.
Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tirupati, Andhra Pradesh.

ABSTRACT

The objective of the present study is to design and to develop a tablet formulation of nano calcium obtained from marine molluscan shells like cut ribbed ark shells are rich source of calcium carbonate with an aim to improve the bioavailability of the drug. These shells were washed and crushed into powder. Approximately 100 g of this powder was treated with various reagents like HCl, acetic acid and salts like sodium bi carbonate. Later it was bound with chitosan in different quantities (1 g, 1.25 g and 1.5 g) to extract pure calcium carbonate. Nano calcium was obtained by keeping the dry extract in muffle furnace and burning chitosan at 650ºC for 2 hrs. This is called decomposition technique. The obtained nano calcium was tested for its chemical composition, purity, SEM analysis, zeta potential analysis and *in vivo* drug release pattern. The results obtained shown that the nano calcium powder particles have the size range of 100-200 nm. Its zeta potential is -30 mV. The purity of nano calcium powder is 99.4%. Nano calcium tablets of five formulations NCT_1, NCT_2, NCT_3, NCT_4 and NCT_5 were prepared using HPMC and starch by direct compression method. These tablets were evaluated for thickness, weight variation, hardness, friability, disintegration time along with *in vivo* drug release studies for 15 min, 30 min and 60 min. The tablets were shown good raise in blood calcium levels but were less than the marketed product. Further optimization of the formula is needed to confirm the results.

KEYWORDS: Nano calcium cut ribbed ark shells, chitosan, SEM, zeta potential, *in vivo* studies, nano calcium tablets, NCT.

INTRODUCTION

The group IIA of the periodic table contains the alkaline earth metals such as Ca, St, Ba and Ra. All the non-metallic substances are insoluble in water and unchanged by five elements were called earths. Limes and magnesia showed alkaline reactions hence called alkaline earths. The name calcium is derived from Latin word '*calas'* meaning lime was known as early as the first centuries when the ancient Romans prepared lime as CaO. It was not actually isolated until 1808 in England when Sir Humphrey Davy electrolyzed a mixture of lime and mercuric oxide. Davy was trying to isolate calcium along with magnesium, strontium and barium. Out of alkaline earth group, Ca has achieved the greatest use and tonnage.

Since from the past 1000 years Pearl powder, a natural product from molluscs used as a rich calcium adjunct by Chinese people to maintain the health of skeleton. Osteoporosis is a worldwide problem associated with its age-related fractures; one of the major reasons for the occurrence of osteoporosis is calcium deficiency. The recommended daily calcium intake is 1000 mg for adult males and females <50 years of age and 1200 mg for those >50 years of age per the Institute of Medicine. The daily calcium intake recommendation by the National Institutes of Health for women are: 1000 mg for premenopausal aged 25 to 50 years and postmenopausal <65 years of age using estrogen; 1500 mg for postmenopausal women not using estrogen and all women >65 years of age. The Canadian recommended total daily calcium intake is 1000 mg for men up to age 50 and premenopausal women and 1500 mg for men over 50 and postmenopausal women.

The uptake of various calcium supplements, including calcium carbonate, calcium citrate, calcium phosphate, calcium gluconate, calcium glubionate, calcium lactate, tricalcium phosphate, calcium gluconolactate, calcium citrate malate, and tricalcium phosphate/calcium lactate were investigated before, Among them calcium carbonate prepared from different varieties of shells like cut ribbed ark shells, oyster shells are the most common ones on the market. Calcium carbonate ($CaCO_3$) is the most abundant mineral in nature, which makes it a cheap, inorganic material. Synthetic forms of $CaCO_3$ have been used in oil, paint, paper, plastics, coatings, environmentally friendly items, calcium-enriched foods, drug delivery, templates for microcapsules, and bone filling material.

Currently there is a wide number of calcium supplement dosage forms especially tablets are available in the market. But all dosage forms include in combination with vitamin D because

optimal calcium absorption may require supplemental vitamin D for those with inadequate vitamin D intake, inadequate sun exposure, or those with impaired renal activation of the vitamin and when recommending calcium supplements, the presence of achlorhydria, use of H2blockers or proton pump inhibitors, number of tablets needed to achieve the desired dose, size of the tablet, formulation, and the cost should all be considered. In patients with low gastric acidity such as the elderly or patients on H2blockers or proton-pump inhibitors (PPI), calcium citrate is a better choice as it is better absorbed in these cases. Calcium citrate is also better absorbed on an empty stomach compared to calcium carbonate.

Nanotechnology is a new technology in many industries including food and pharmacy. The characteristics of materials change significantly in nanonization. For example, nanonization may improve the absorption rate of drugs or nutrients in humans, resulting from an enlarged specific surface area and an increased solubility of the particle could enhance the bioavailability substantially. In the present study, we made an attempt to prepare nano calcium powder by using cut ribbed arc shells and its compression to formulate nano calcium tablets and also evaluated the suitability of nano particle based calcium carbonate tablets for its preparation of nano calcium, characterization of nano calcium, *in vivo* drug release studies, preformulation studies, compression of nano calcium tablets and evaluation of prepared nano calcium tablets.

MATERIALS AND METHODS

Materials

Cut ribbed arc shells (marine source) were obtained from the near sea shore area Nellore, All reagents such as concentrated Hcl, glacial acetic acid, sodium carbonate and chitosan, HPMC (hydroxyl propyl methyl cellulose), Starch, talc used in this study were of reagent grade from Himedia and the instruments used in this study such as Digital Weighing balance (Shimadzu Corporation, Japan), FTIR Spectrophotometer (Analytical 2202, Mumbai), Muffle furnace (Tempo instruments and equipment's PVT.LTD), UV/Vis Spectrophotometer1800(Shimadzu Corporation, Japan), Hot Air Oven (Hasthas), SEM analyzer(JEOC-JSM 5800), Zeta potential analyzer(Horiba Scientific Sz-100), Auto analyzer(MISPA EXCEL(491209018 EGE2), Tablet compressing machine(Rimek mini press (16 station)), Monsanto Hardness tester(INCAB Tablet Hardness Tester), Vernier callipers Tester(ABSOLUTE DIGIMATIC), Disintegration apparatus(ED-2L/ELECTRO LAB).

Preparation of nano calcium[24-27]

Processing of cut ribbed ark shells: Approximately 10 g of cut ribbed ark shells were weighed and washed with tap water in order to remove dirt from outer surface and inside of the shells.

Drying: The shells were boiled for 30 min by using steel container and dried in a hot air oven at 110ºC for 2days. The dried cut ribbed ark shells were finely ground using a grinder.

Synthesis of Nano calcium

- The nano sized calcium carbonate was synthesized by an *in-situ* decomposition technique. The 1g powder of calcium carbonate was dissolved in about 0.75 ml of Conc. hydrochloric acid.

$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2$

- The obtained calcium chloride solution is mixed with chitosan. Various proportions of Chitosan (NC_1-1 g, NC_2-1.25 g and NC_3-1.5 g) were dissolved in 3% acetic acid. The different ratios of chitosan were being used to identify the better concentration of chitosan in the synthesis of nano calcium.

Table No 1: Working Formula for the preparation of Nano calcium powder.

Ingredients	NC_1	NC_2	NC_3
$CaCO_3$ (g)	1	1	**1**
Chitosan (g)	1	1.25	**1.5**
HCl (ml)	0.75	0.75	**0.75**
Na_2CO_3 (g)	0.322	0.322	**0.322**

- This mixture was blended with 0.322 g of sodium carbonate solution and slightly heated the mixture to complete the reaction.

$CaCl_2 + Na_2CO_3 \rightarrow CaCO_3 + 2\ NaCl$

- The mixture was kept overnight.
- Obtained calcium carbonate precipitate was filtered off, washed several times with water to remove sodium chloride, hydroxide etc. and dried.
- It was kept for 2 hrs calcinations in a muffle furnace at 650ºC to burn Chitosan and to obtain nano sized calcium carbonate nanoparticles.

The obtained powder was stored in small air tight bags to avoid absorption of any moisture.

Characterization of nano calcium

Identification tests of calcium carbonate[28]: 0.5 g of powder was dissolved in 8 ml of 2M acetic acid. When effervescence ceases, the solution was boiled for 2 min, cooled and then diluted to 10 ml with 2M acetic acid. The impurities were removed by means of filtration process.

Calcium

a. 20 mg of sample was taken. 5 ml of 5M acetic acid and 1 ml of glacial acetic acid were added and dissolved completely. Then 0.5 ml of potassium Ferro cyanide solution and 50 mg of ammonium chloride were added.
b. 5 ml of 0.4% w/v solution of sample was taken and to this solution, 0.2 ml of 2% w/v solution of ammonium oxalate was added.
c. 20 mg of sample was taken and dissolved in HCl and was neutralized with dilute NaOH. 5 ml ammonium carbonate solution was added to this.

Carbonate

a. 0.1 g of sample in a test tube was suspended in 2 ml of water. 2 ml of 2M acetic acid, was added and closed the test tube. The suspension was heated gently and collected the gas in 5 ml of 0.1M barium hydroxide.
b. Solution of substance was mixed with magnesium sulphate.

Assay of nano calcium carbonate[28]: 0.1 g of substance was taken in a conical flask and dissolved in 3 ml dilute HCl and 10 ml of water. It was boiled for 10 min and then cooled and then diluted to 50 ml with water. 8 ml of sodium hydroxide few drops of calcon were added to this mixture. This mixture was titrated with 0.05M of disodium edetate until the solution changes from pink to full blue colour.

Each ml of 0.05M disodium edetate ≈ 0.005004g of calcium carbonate

In order to compile the purity standards as per IP, the percentage purity of calcium carbonate should be not less than 98% and not more than 100.5%.

SEM analysis: The $CaCO_3$ nano powder was affixed to a metallic stub which is placed on the sample holder. The sample holder was then fixed on a rotatable disc inside the machine. The surface morphology of the powder sample was observed on SEM (JEOL-JSM 5800)

operated under low vacuum at an accelerating voltage of 20 kV to get the sharp image of the sample.

Zeta potential analysis[29]

The sample of $CaCO_3$ nano powder was suspended in water. The viscosity of the obtained mono dispersion medium was 0.89 mPa.s. This suspension was analyzed for the particle size and zeta potential at 25.2 °C temperature, 173 scattering angle, 0.293 Ms/cm conductivity, 3.3 electric voltages by placing the sample in the zeta potential analyzer (Horiba Scientific Sz-100).

***In vivo* drug release studies of nano calcium**

Selection of animals: Albino rats of either sex weighing 150-225 g were used for the study after obtaining the approval of the Institute's Animal Ethics Committee (approval code no: SPSP: 1016/PO/E/S/CPCSEA/2016/010).

Animals were divided into three groups. Prior to the experiment animals were kept under fasting for 18 hours. On the day of the experiment the animals were administered with test and the standard compounds of dose 500 mg/kg; *p.o*. After administration of Test and standard compounds blood samples were withdrawn through retro-orbital puncture in rats at an intervals of 15, 30, 60 minutes respectively. The blood samples were centrifuged and the serum was separated. The serum which was separated is used for calcium estimation by using commercially available kits (Auto Span Diagnostic kits).

Estimation of Calcium

Method: O-Cresolphthalein Complexone, End Point Assay (Barnett R.N *et al.*, 1973)

Principle: In alkaline solution, Calcium binds with metal complexing dye O-Cresolphthalein Complexone (OCPC) to form a bluish purple complex, which is measured at 578nm. The intensity of color formed is proportional to calcium concentration in the sample. Hydroxyquinoline will act as a masking agent and eliminate the interference of magnesium.

Calcium + OCPC → Bluish-purple colour

Reagents

1. OCPC Reagent
2. AMP Buffer
3. Calcium Standard

Working reagent Preparation

1. Reagent 1 was diluted with Reagent 2 in equal proportion i.e. 1 ml of Reagent1 + 1ml of Reagent 2. These were mixed properly by gentle swirling.
2. Thus reagent 3 was ready to use

Assay parameters

Table no 2: Assay parameters.

Mode	End point
Wavelength	578nm
Blanking	Distilled water
Flow cell temperature	37^{o}C
Optical path length	1cm
Permissible Reagent Blank absorbance	<0.5 AU
Standard Concentration	10 mg/Dl
Units	mg/dl

Procedure

Table no 3: Volumes of test and standards employed.

Pipette in to tubes marked	Blank	Standard	Test
Sample	-	-	20 µl
Reagent 3		20µl	-
Working calcium Reagent	1000 µl	1000 µl	1000 µl

The reagents were mixed well and incubated at 37^{o}C for 5 minutes.

The analyser was programmed as per assay parameters.

1. Distilled water was used as blank.
2. Absorbance of standard followed by the test was measured.
3. The results were calculated as per given calculation formula.

Calculation

$$\text{Calcium concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. Of Standard}$$

Note: Normal range of calcium is 5.23 mg/dl.

The calcium concentration of test and standard at 30, 60 and 90 minute's time.

Preformulation studies

Preformulation studies are performed to investigate the physical and chemical properties of a drug substance alone and also when combined with other substances such as excipients. It is the first step in the rational development of dosage forms.

Objective and Scope

The objective of performing preformulation testing is to generate information that will be helpful in developing a stable and bio-available dosage form when combined with excipients. The use of Preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product and at same time provides the basis for optimization of the drug product quality.

Drug Excipient compatibility studies by force degradation studies: Drug is in intimate contact with one or more excipients in all the dosage forms. Later it could affect the stability of drug. Knowledge of drug-excipients interaction is useful in selecting an appropriate Excipient.

Procedure: The mixtures of drug and excipients (1:1) were prepared, and packed in both closed vials and kept in both long term and accelerated environmental conditions (25°C/60% RH and 40°C/75% RH) for 1 month. At the end of 1 month period all the samples were observed physically**.**

Angle of repose: The flow property was determined by measuring the Angle of Repose. It is the maximum angle that can be obtained between the free standing surface of a powder heap and the horizontal plane. Such measurements give at least a qualitative assessment of the internal cohesive and frictional effects under low levels of external loading, as might apply in powder mixing, or in tablet die or capsule shell filling operations.

$$\theta = \tan^{-1}$$

Where,

h = height

r = radius

θ = angle of repose

Table No 4: Flow Characteristics.

Angle of repose	Properties
<25	Free flowing
25-30	Good
30-40	Fair
>40	Poor

Procedure

- An accurately weighed sample was taken.
- A funnel was fixed in the stand in such a way that the tip of the funnel was at the height of 6 cm from the surface.
- The sample was passed through the funnel slowly to form a heap.
- The height and the circumference of the powder heap formed were measured.
- The radius was measured and the angle of repose was determined using the above formula. This was repeated three times for a sample.

Determination of bulk density and tapped density: A quantity of 10 g of the powder (W) from each formula was introduced into a 50 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 sec intervals. The tapping was continued until no further change in volume was noted.

The bulk density and tapped density were calculated using the following formulas

$$\text{Bulk density} = W / V_O$$

$$\text{Tapped density} = W / V_f$$

Where, W = weight of the powder,

V_O = initial volume,

V_f = final volume.

Hausner's Ratio: It indicates the flow properties of the powder and is measured by the ratio of tapped density to the bulk density.

Hausner's Ratio = Tapped density/Bulk density

Table No 5: Limits of Hausner's Ratio as per USP.

S. NO	HAUSNER'S RATIO	PROPERTY
1.	Less than 1.25	Free flow
2.	1.25 to 1.5	Glidant to be added
3.	More than 1.5	Poor flow

Compressibility index (Carr's index): Compressibility index is an important measure that can be obtained from the bulk and tapped densities. In theory, less the compressibility of a material, it is more flowable. A material having values of less than 20 to 30% is defined as the free flowing material.

$C_I = 100 (V_O - V_f)/V_O$

Where C_I is carr's index

V_O is intial volume

V_f is final volume

Table No 6: Flow Characteristics.

% Comp. Index	Properties
5-12	Free flowing
12-16	Good
18-21	Fair
23-35	Poor
33-38	Very poor
>40	Extremely poor

Preparation of Nano calcium tablets

Table No 7: Formulation of nano calcium tablets.

Formulation	NCT_1	NCT_2	NCT_3	NCT_4	NCT_5
$CaCO_3$ nano powder (mg)	110	110	**110**	110	110
HPMC (mg)	70	65	**77**	65	60
Starch (mg)	90	97	**85**	100	98
Talc (mg)	Qs	Qs	**Qs**	qs	Qs

- The nano calcium tablets were prepared by direct compression method. All the ingredients were weighed accurately according to the formula and are passed through #30 mesh
- All the sifted ingredients were mixed uniformly and sufficient quantity of talc was added at the end stage of mixing.
- The powder mixture was compressed into tablets using Rimek mini press (16 station) machine.
- The compressed tablets were stored for further use and analysed for physical strength and dimensions.

Evaluation of nano calcium tablets[30]

Physical Appearance: The general appearance of a tablet, its identity and general elegance is essential for consumer acceptance, for control of lot-to-lot uniformity and tablet-to-tablet uniformity. The control of general appearance involves the measurement of size, shape, colour, presence or absence of odour, taste etc.

Thickness: It can be dimensionally described and controlled. The thickness of a tablet is only variable. Tablet thickness was measured by screw guaze. Tablet thickness should be controlled within a ± 5% variation of standard value.

Weight Variation test: 20 tablets were taken and weighed individually. The average weight was calculated and compared with the individual weight of the tablet. The tablet pass IP if not more that 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit.

Hardness: Tablet requires a certain amount of strength or hardness and resistance to friability to withstand mechanical shakes of handling in manufacture, packaging and shipping. Hardness generally measures the tablet crushing strength.

Friability: Friability of a tablet can determine in laboratory by Roche friabilator. This consist of a plastic chamber that revolves at 25 rpm, dropping the tablets through a Distance of six inches in the friabilator, which is then operate for 100 revolutions. The tablets are reweighed. Compress tablet that lose less than 0.5 to 1.0 % of the Tablet weigh are consider acceptable.

Disintegration time: The disintegration of tablets was carried by using tablet disintegration test apparatus in 900 ml of water at 37°C and were studied for their disintegration time.

***In vivo* drug release studies of nano calcium tablets:** Albino rats of either sex weighing 150-225 g were used for the study after obtaining the approval of the Institute's Animal Ethics Committee (approval code no: SPSP: 1016/PO/E/S/CPCSEA/2016/010).

The tablets were triturated into powder using a mortar and pestle. The amount of powder to be fed was calculated according to the weight of the rats and was fed with little addition of water using a syringe. The test was carried same as that of the *invivo* drug release of nano calcium.

RESULTS AND DISCUSSION

Preparation of Nano calcium

Calcium carbonate nano particles were prepared by using 3 combinations of chitosan viz. 1 g, 1.25 g and 1.5 g of chitosan. Out of these three samples, the nano calcium prepared with 1.5 g of chitosan had shown good results in the physical examination. In the remaining two

samples, due to insufficient ratio of chitosan, charring of the powder in the muffle furnace occurred. So the sample prepared with 1.5 g chitosan was preceded for further analysis.

Characterization of Nano calcium

Identification of calcium carbonate

Calcium

Table no 8: Identification test results of calcium.

Test	Observation	Inference
a.	A white crystalline precipitate was formed.	Confirmed the presence of calcium.
b.	A white precipitate was formed which was soluble in HCl and sparingly soluble in dil. acetic acid.	Confirmed the presence of calcium.
c.	A white precipitate was formed which was sparingly soluble in ammonium chloride.	Confirmed the presence of calcium.

Carbonate

Table no 9: Identification test results of carbonate.

Test	Observation	Inference
a.	A white precipitate was formed which was dissolved in excess of dil. HCl.	Confirmed the presence of carbonate.
b.	A white precipitate was formed.	Confirmed the presence of carbonate.

Assay of calcium carbonate

From the observed data, Nano calcium carbonate was found to be 99.4% pure and is within the limits of purity standards.

SEM analysis

The SEM images of nano calcium showed that the particles are having the size range within the limits of 100-200 nm. The particles were irregular in shape.

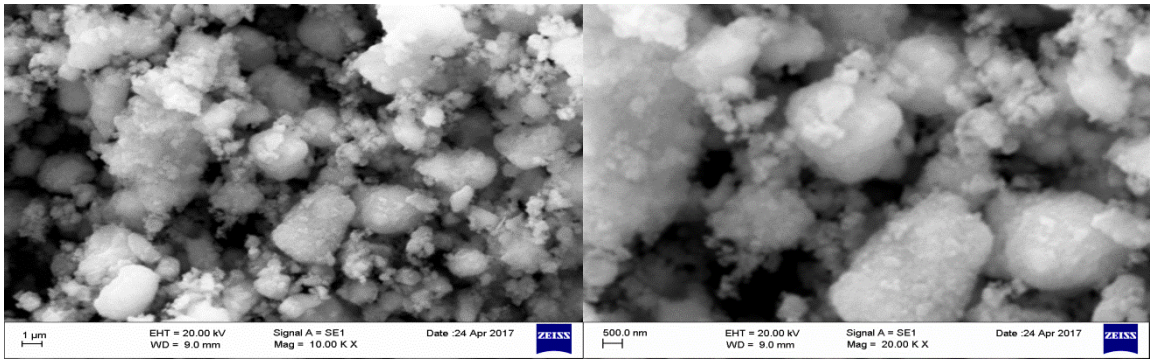

Fig no 1: SEM morphology of Nano calcium powder.

ZETA potential analysis

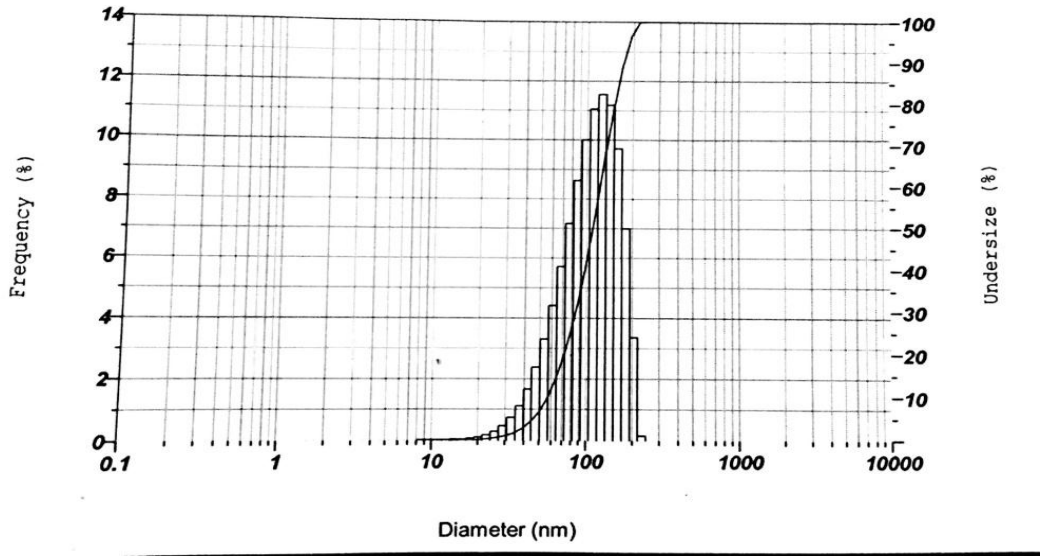

Fig no 2: Particle size analysis.

The results of particle size analysis had shown that the mean particle diameter was 20.0nm with 43.3nm standard deviation.

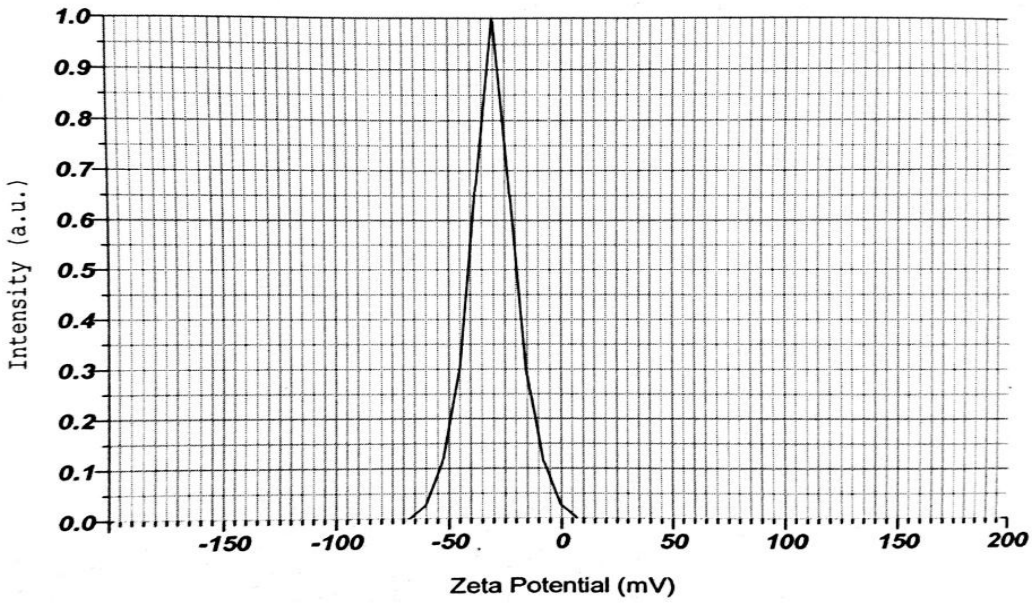

Fig no. 3: Zeta potential analysis.

The zeta potential of the Nano calcium particles were found to be -31 mV indicating that the particles having negative zeta potential. Since these particles possess optimum zeta potential, the particles will be more stable.

Preformulation studies

Preformulation studies were first step in rational development of dosage form of a drug substance. The objectives of preformulation studies were to develop a portfolio of information about drug substance, so that this information was useful to develop formulation. Preformulation can be defined as investigation of physical and chemical properties of drug substance alone and when combined with excipients.

Drug Excipient compatibility studies by force degradation studies

Table no 10: Drug Excipient compatibility studies by force degradation studies.

Drug + Excipient	Initial	First month Observation		Compatible
		25ºC/60% RH	40ºC/75% RH	
Nano calcium	White Powder	No change	No change	Yes
Nano calcium + HPMC	Creamy White	No change	No change	Yes
Nano calcium + starch	White Powder	No change	No change	Yes
Nano calcium + talc	White Powder	No change	No change	Yes

Flow properties

Table no 11: Flow characteristic of NCTs mixture.

S.No	Formulation code	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose	Carr's Index	Hausner's ratio
1	NCT_1	0.425	0.476	24.84	12.0	1.12
2	NCT_2	0.416	0.476	27.96	14.4	1.14
3	NCT_3	0.408	0.470	30.25	15.1	1.15
4	NCT_4	0.384	0.434	25.58	13.0	1.13
5	NCT_5	0.370	0.430	28.17	16.2	1.16

From the study of bulk density, tapped density, angle of repose, carr's index and hausner's ratio, it was found that all the formulation mixtures for NCTs have good flow properties.

Evaluation of Tablets

Table no 12: Evaluation of NCTs.

S.No	Formulation code	Thickness (mm)	%Weight variation	Hardness (kg)	%Friability	Disintegration time (min)
1	NCT_1	1.41	0.31	4.3	0.53	14
2	NCT_2	1.42	0.30	4.6	0.49	15
3	NCT_3	1.44	0.22	4.2	0.51	16
4	NCT_4	1.43	0.31	4.5	0.49	13
5	NCT_5	1.44	0.29	4.5	0.55	14

Thickness: All the tablets of all 5 formulations were shown no variation in the size and shape and were within the limits.

Weight Variation test: All the 5 formulations were passed the weight variation test.

Hardness: All the 5 formulations were having the according to the specification limits.

Friability: All formulations were passed the friability test.

Disintegration time: Almost all the tablets of 5 formulations were disintegrated within 15 min.

***In vivo* drug release studies of nano calcium**

Table no 13: Blood plasma levels of NCTs.

S.No	Formulation code	Calcium Level (mg/dl)		
		15 min	30 min	60 min
1	NCT_1	6.6	8.4	11.6
2	NCT_2	6.1	8.0	10.8
3	NCT_3	**6.7**	**9.0**	**12.9**
4	NCT_4	6.4	8.4	11.3
5	NCT_5	6.1	7.9	10.2

From the data obtained from animal studies, it was found that all five formulations shown more than 10 mg/dl within 1 hour and formulation NCT_3 shown 12.9 mg/dl of calcium.

Table no 14: Blood calcium levels of animal groups.

S .No	Groups	Calcium Level (mg/dl)		
		15 min	30 min	60 min
1	Normal	5.4	5.4	5.4
2	Nano calcium powder	**7.9**	**9.8**	**13.5**
3	Nano calcium tablets	6.7	9.0	12.9
4	Marketed tablets	7.5	9.2	13.2

From the analysis of blood plasma levels of all the animal groups (no drug, nano calcium powder, nano calcium tablets and marketed product), it was observed that all groups have shown increased calcium levels compared to the normal. Though the levels of calcium for the nano powder were more when compared to marketed product, the nano calcium tablets have shown a slight decrease to the marketed product. Henceforth further optimization of formula is needed. As a whole the *in vivo* drug release studies proved that the decreased size of calcium carbonate has its influence on the calcium absorption.

CONCLUSION

Nano sized calcium carbonate drug particles can be prepared from the cut ribbed ark shells (marine source) by using chitosan. The obtained nano calcium carbonate was 99.4% pure and was met the purity standards as per IP. These Nano particles have the size range between 100

to 200 nm. The zeta potential of these particles was -31 mV indicating that the particles were stable and doesn't tend to form aggregates. The *in vivo* studies of nano calcium powder were shown a significant increase in the blood calcium levels compared to the marketed product. The synthesized nano calcium powder was compressed into tablets of five formulations using HPMC, starch in varying compositions (NCT_1, NCT_2, NCT_3, NCT_4 and NCT_5). All five formulations passed the general evaluation tests like thickness, weight variation, hardness, friability and disintegration time. Though the levels of calcium for the nano powder were more when compared to marketed product, the nano calcium tablets have shown a slight decrease to the marketed product. Henceforth further optimization of formula is needed. As a whole, the *in vivo* drug release studies proved that the nano size of calcium carbonate has its influence on the calcium absorption.

REFERENCES

1. Huang S, Chen JC, Hsu CW, Chang WH. Effects of Nano calcium carbonate and Nano calcium citrate on toxicity in ICR mice and on bone mineral density in an ovariectomized mice model. *Nanotech*, 2009; 16: 20(37): 375102.
2. H.S. Chen, J.H. Chang, and J.S.B. WU calcium bioavailability nanonized pearl powder pearl powder for others. *Journal OF food science*, 2008; 73: 9.
3. Diane Render, Temesgen Samuel, Howard King, Madan Vig, Shaik Jeelani, Ramapuram Jayachandra Babu, and Vijaya Rangari. March 2016.
4. Neethumol Varghesel, Manjusha Harifaran, Benny cherian A. Synthesis of calcite nanoparticles from biogenic waste using gelatin and its spectral characterization. *World J of Pharm Res*., 2015; 4(12): 1302-1313.
5. Takuya Tsuzuki, Kellie Pethick, Paul G, McCormick. Synthesis of $CaCO_3$ Nano particles by mechanochemical processing. *J Nanoparticle Res.*, 2000; 2: 375-380.
6. Nishikant c sinde: A review article on Nanoparticles –Advances in drug delivery system. *Res J Pharm, biological chem.* 2012, ISSN: 0975-8585, Page No. 922.
7. Kavitha V et al. A review article on Nanosuspension Formulation: An Improved Drug Delivery System. *Nanosci tech.* 2014, ISSN: 2278-1374; 4(1): 1-5.
8. Fuad MYA, Hanim H, Zarina R, Mohd ZA. Ishak, Azman Hassan. Polypropylene/calcium-carbonate Nanocomposites – effects of processing techniques and maleated polypropylene compatibiliser. *eXPRESS Polymer LetterS*, 2010; 4(10): 611–620.

9. Limin Sun, Laurence C. Chow, Stanislav A, Frukhtbeyn, John E Bonevich. Preparation and Properties of Nanoparticles of Calcium Phosphates with Various Ca/P Ratios. *J Res NatlInst Stand Technol,* 2010; 115(4): 243-255.
10. Nagavarma B V N, Hemant K.S.Yadav, Ayaz A, Vasudha LS, Shivakumar HG. Different techniques for preparation of polymeric nanoparticles- a review. *Academic Sci.,* 2012; 5(3): 16-23.
11. SovanLal Pal, Utpal Jana, Manna PK, Mohanta GP, Manavalan R. Nanoparticle: An overview of preparation and characterization. *J Applied Pharm Sci*., 2011; 1(6): 228-234.
12. Leon Lachman, Joseph L. Kanig et al, Tablet introduction, 1987; (3): 293-294.
13. Ansel's by pharmaceutical dosage forms & drug delivery system by Loyd V Allen, Nicholas G Popovich, (8): 227-245.
14. IP 1996, volume I: 126.
15. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of Pharmaceutical Excipients, 6th edition. *Pharm Press, UK.,* 2009: 86-89.
16. Goosen, Mattheus FA. (1 June 1996). Applications of Chitan and Chitosan. *CRC Press*. 1996: 132-139.
17. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of Pharmaceutical Excipients, 6th edition. *Pharm Press, UK*. 2009: 159-161.
18. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of Pharmaceutical Excipients, 6th edition. *Pharm Press, UK*. 2009: 308.
19. https://pubchem.ncbi.nlm.nih.gov/compound/hydrochloric_acid.
20. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of Pharmaceutical Excipients, 6th edition. *Pharm Press, UK*. 2009: 635-636.
21. https://pubchem.ncbi.nlm.nih.gov/compound/sodium_carbonate.
22. Raymond C Rowe, Paul J Sheskey, Marian E Quinn. Handbook of Pharmaceutical Excipients, 6th edition. *Pharm Press, UK*. 2009: 5-6.
23. https://pubchem.ncbi.nlm.nih.gov/compound/acetic_acid.
24. IP 1996, volume I: 126.
25. IP 1996, volume II: 126.
26. Manjusha Hariharan, Neethumol Varghese, *et al*., Synthesis and Characterisation of $CaCO_3$ (Calcite) Nano Particles from Cockle Shells Using Chitosan as Precursor. *Int J SciRes Publications,* 2014; 4(10): 1-5.

27. Krishna Sailaja A, Amareshwar P, Chakravarty P. Different techniques used for the preparation of nanoparticles using natural polymers and their application. *Int J Pharmacy Pharm Sci.,* 2011; 3(2): 45-50.
28. Oladoja NA, Ololade IA, Olatujoye VO, Akinnifesi TA. Performance Evaluation of Fixed Bed of Nano Calcium Oxide Synthesized from a Gastropod Shell (*Achatinaachatina*) in Hexavalent Chromium Abstraction from Aqua System. *Water, Air, & Soil Pollution,* 2012; 223(4): 1861-1876.
29. Samiksha Wasnik, Avijit Guha, Arvind Sinha, Shashi Singh. Evaluation of nano biphasic calcium phosphate ceramics for bone tissue engineering applications: *In vitro* and preliminary *in vivo* studies. J Bio materials Applications, 2012; 27(5): 565-575.
30. Reddy S, Wasnik S, Guha A, Kumar JM, Sinha A, Singh S. Evaluation of Nano-biphasic calcium phosphate ceramics for bone tissue engineering applications: *in vitro* and preliminary *in vivo* studies. J Biomater Appl., 2013; 27(5): 565-75.
31. Jeffrey D. Clogston. Measuring zeta potential of Nano particles. *Nanotech characterization lab,* 2009.
32. IP 1996, volume II, appendix 3.1: 35-36.
33. IP 1996, volume I: 126.
34. Fadi F. Siyam, David M. Klachko. What Is Hypercalcemia? The Importance of Fasting Samples. *Cardiorenal Med*. 2013; 3(4): 232–238.
35. Ansel's by pharmaceutical dosage forms & drug delivery system by Loyd V Allen, Nicholas G Popovich, (8): 227-245.
36. V.Manimaran et al tablet ingridients, 2016; 4(5): 7-15.
37. Modern pharmaceutics edited by Gilbert S Banker, Christopher T Rhodes, (4): 287-329.