FORMULAS COMPRISING HIGHLY SOLUBLE ELEMENTS AND VITAMIN FOR THE PREVENTION AND AMELIORATION OF OSTEOPOROSIS

[0001] This application claims the benefit of priority of Taiwanese Application No. 100126601, filed July 27, 2011, and U.S. Application No. 61/512,685 filed July 28, 2011. The entire contents and disclosures of the preceding applications are incorporated by reference into this application.

[0002] Throughout this application, various references are referred to and disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

[0003] Calcium is the major element in bones with over 99% of the body's calcium existing in bone. Adequate intake of calcium from the diet is necessary for bone growth and maintenance. Osteoporosis is a disease caused by a significant loss of bone mass leading to increased susceptibility to fracture, most often occurring in women age 35 or above, but more frequently, occurring in postmenopausal women (1, 2).

[0004] Dietary supplements with calcium were thought to be primary to maintaining bone health in the past 50 years (3). However, the benefit of increased overall calcium consumption on bone health has not been clearly demonstrated, and there are conflicting reports in the literature on its effectiveness. In 43 studies of calcium supplementation reviewed by Heaney published between 1988 and 1993, 16 of the 19 placebo-controlled studies in which calcium intake was controlled showed that the mineral prevented or slowed bone loss, but 16 studies showed that calcium had no effect on bone loss (4, 5).

[0005] In the 12 studies that excluded women who were within 5 years of menopause, a period when estrogen deficiency overwhelms the effect of calcium supplementation (6), all showed that calcium had a significant beneficial effect.

[0006] In elderly women, it was shown that there was a significant relationship between bone mineral density (BMD) and several critical nutrients: energy, protein, calcium, magnesium, zinc and vitamin C (1). It has also, however, been found that high levels of calcium intake may be linked to higher incidence of cardiovascular disease (3).

[0007] Since total calcium intake has not shown to be conclusive with respect to bone health, other factors have also been taken into account, such as the calcium to magnesium ratio in modern diets, and in supplement form. The ratio of Ca/Mg in the modern diet increased from 2/1 in the first 40 years of the 1900s to > 3/1 in the 1960s, to > 6/1 in the year 2000. The daily recommended intake (DRI) in the year 2000 of Ca/Mg was > 3/1 to > 4/1. This change correlates with negative consequences with respect to bone health as well as an increased risk of cardiovascular disease.

[0008] It should be noted that the increase in Ca/Mg is mainly due to the increase in calcium intake, not a change in magnesium. In the early 2000s, daily calcium intake reached a new high of 2,500 mg (3). The daily requirement of calcium was recently re-evaluated (7). It was found that an average intake of 749 mg of calcium is required, an estimate lower than previously estimated.

[0009] Supporting the thesis that Ca/Mg ratio, among other factors, is a more important factor in bone maintenance and health than absolute calcium consumption, is one clinical trial, wherein 43 early postmenopausal women were randomly assigned to one treatment group for administration of the following: percutaneous estradiol, oral calcium (2000 mg/day) or placebo. Bone mineral content in the forearm, the entire body and spine remained the same in the estradiol group; however, there was a decline in the calcium and placebo groups. Calcium did not show any significant effect and calcium supplementation may have a minor effect on the loss of cortical bone, but it had no effect on the trabecular bone (6).

[0010] In a National Health and Nutritional Examination Survey (NHANES) conducted from 1988 to 1994, predictive models were established to evaluate parameters such as race, body composition, exercise, alcohol intake, smoking status and nutritional intake (8). The nutritional intake analysis included study of elements such as calcium, phosphorus, magnesium, iron, zinc, sodium and potassium. Among the 7,532 women in the study who were 20 years or older, elemental intake was not a predictor of osteoporosis. However, the average calcium intake was 659 mg and magnesium was 241 mg - lower than that of the RDA of 1000 and 310 mg, respectively.

[0011] Physical activity was associated with increase in vertebral bone mineral density (9). When activity was removed, vertebral bone mineral density was dependent on calcium intake. The relationship disappeared when calcium intake exceeded 800 to 1000 mg/day. A ceiling effect of calcium was also observed by Celotti and Bignamini (10). They reported that calcium

supplementation is important for maintaining bone health. However, an excessive amount of calcium may be useless and could cause hypercalciuria and kidney stones. Supplementation with a small amount of magnesium was suggested.

[0012] Other studies show not just the importance of the ratio of Ca/Mg consumed or administered, but the importance of optimizing zinc levels. Mutlu et al. (11) showed that magnesium and zinc levels are the lowest in postmenopausal women, lower than postmenopausal women with osteopenia, and lower than postmenopausal women with normal bone density. Calcium supplementation may reduce zinc absorption, and magnesium and zinc retention. Consequently, calcium supplementation in the absence of the administration of other optimized amounts of minerals may further aggravate the severity of osteoporosis (2, 12, 13). Apart from calcium, magnesium, zinc, manganese and copper deficiencies are linked to osteoporosis (14).

[0013] Angus et al. (15) showed that calcium was not a predictor of bone mineral density in pre- and post-menopausal women. Magnesium and iron were, however, predictors of bone mineral density. In this study, however, the test subjects ingested less than the recommended amounts of elements. About 29% of the post-menopausal women consumed less than 500 mg of calcium per day (16), while other nutrients such as magnesium, etc. were also deficient.

[0014] A study emphasizing the benefit of magnesium on postmenopausal women found that a Mg/Ca ratio of 1.2/1 was more effective at maintaining bone health than that of a ratio of 0.4/1 (17). The study used 500 mg of calcium in the form of calcium citrate and 200 mg of magnesium in the form of magnesium oxide for the 0.4/1 group and 600 mg of magnesium in the form of magnesium oxide in the 1.2/1 group. The study showed that women on the 1.2/1 diet for 6 to 12 months had an average of an 11% increase in bone mineral density, whereas, the other group had a non-significant increase of 0.7%.

[0015] Although bone health is dependent on a variety of factors, there is enough evidence to show that, in the area of elemental requirements, apart from calcium, other elements such as magnesium, phosphorus, zinc, copper, etc. are also important for maintaining or improving bone health. Further, due to differences in bioavailability, it is proposed that elemental salts would be more accurately characterized in terms of absorbability, and that calcium formulas be optimized through the use of preferred salts.

[0016] The selection of appropriate salts for optimized formulations has not received appropriate attention because of reports showing that solubility of calcium salts is not related to the element's bioavailability. The absorption of calcium salt, soluble or insoluble, is not affected by gastric acid secretion (18). The hypothesis that calcium carbonate can be converted to a more soluble calcium salt in the stomach, namely calcium chloride, thus enhancing calcium absorption, has been tested. The results showed that calcium carbonate absorption is not influenced by gastric acid (18). The average amount absorbed in humans is 24%.

[0017] The bioavailability of calcium carbonate, D-calcium lactate, L-calcium lactate and oyster shell calcium was found to be independent of the salt's solubility (19). This study used a method which was different from that of the balance study. It measured changes in the pituitary thyroid hormone (PTH), etc. instead of actual calcium absorption. However, indirect methods of measurement, such as PTH, do not provide truly accurate comparisons of calcium bioavailability.

[0018] Using Ca^{45} as a tracer, fractional absorption values of calcium carbonate and calcium citrate were found to be insignificantly different from each other at a low dose (300 mg calcium); however, calcium absorption from calcium carbonate was slightly but significantly better than calcium citrate (20). Heaney (21) reported that the rates of urinary excretion for three marketed calcium products (marketed calcium carbonate, encapsulated calcium carbonate and marketed calcium citrate) were identical.

[0019] Despite these observations, there are reports showing that not all calcium salts have the same bioavailability. Bioavailability of calcium ascorbate is higher than that of calcium carbonate and calcium chloride (22).

[0020] Bioavailability of calcium acetate was measured using ^{45}Ca (23). Compared to calcium ascorbate, bioavailability of calcium acetate was significantly lower (70% vs 45% at 25 mg calcium load). A kinetic model consisting of 8 compartments was used to fit the plasma calcium vs. time data. The difference was attributed to a saturable process. It is also reasoned that the solubility of calcium acetate may be reduced in the intestine because calcium from the acetate salt may precipitate phosphate or chloride ions in the intestine. Therefore, it is not surprising that the bioavailability of calcium acetate is not different from that of calcium chloride and calcium phosphate.

[0021] Magnesium absorption from 10 organic and inorganic salts was tested in rats (24). The bioavailability of magnesium ranged from 50 to 66%. Magnesium gluconate provided the highest value. The solubility of these salts in the small and large intestine and cecum was also measured. Solubility of these salts was quite high at the proximal section of the intestine; it dropped off very quickly as pH increased along the intestinal tract. Differences in absorption of these magnesium salts may not be important considering the variability among individuals.

[0022] Zinc absorption occurs throughout the small intestine and it is dose dependent in humans (25). With respect to zinc, there was no difference in the bioavailability of zinc oxide and zinc sulfate as measured using dual isotope techniques (26); both were at approximately 24%. The bioavailability of iron was 15.9%. However, zinc sulfate tended to reduce the bioavailability of iron to 11.5% and this number is significant. Eight to 11 mg of zinc per day is the recommended intake (http://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/). The recommended daily allowance of zinc was 6 mg (27).

[0023] The following are inventions and disclosures noteworthy in the art:

[0024] U.S. Patent 5,879,698 issued in 1999 for a calcium dietary supplement comprising calcium, magnesium, zinc, etc. (28). The calcium to magnesium ratio is high and the range of magnesium used was between 50 to 150 mg. The salt for calcium is calcium carbonate. The quantity of calcium and magnesium used and the type of salts employed are different from the present invention.

[0025] U.S. Patent 6,716,454, awarded to Meignant and Stenger in 2004, cites a composition which consists of calcium and a vitamin D mixture.

[0026] U.S. Patent 6,790,462, awarded to Hendricks in 2004, describes a dietary supplement containing calcium and phosphorus. Vitamins including vitamin D could also be included in the supplement. Hendricks emphasized the effects of phosphorus, and optionally vitamins B_{12}, folate and Vitamin B_6. The present application, however, does not include phosphorus.

[0027] Mazer et al. were granted U.S. Patent 5,698,222 in 1997 on a calcium supplement in solid form which contains calcium glycerophosphate, vitamin D and vitamin C. The present invention does not contain calcium salt of this kind.

[0028] In another patent, U.S. Patent 5,075,499, issued in 1991, Walsdorf et al. described the synthesis of dicalcium citrate-lactate by mixing stoichiometric mixtures of citrate and lactate salts to produce the calcium salt (29).

[0029] Krumhar and Johnson designed a diet supplement for bone health, disclosed in U.S. 7,029,703 which issued in 2006, consisting of microcrystalline calcium hydroxyapatite, protein (mostly collagen), phosphorus, fat, and other minerals. It also contains vitamin D_3, cholecalciferol, and a preferred osteoblast stimulant, ipriflavone. In addition to these basic ingredients, the composition can further include various other minerals known to occur in bone, vitamin C, and glucosamine sulfate, all of which have been claimed to have beneficial effects on the growth and maintenance of healthy bone.

[0030] Sultenfuss, in U.S. Patent 5,514,382, issued in 1996, described another daily vitamin and mineral supplement for women comprising vitamin A, beta-carotene, niacin, riboflavin, pantothenic acid, pyridoxine, cyanocobalamin, biotin, para-aminobenzoic acid, inositol, choline, vitamin C, vitamin D, vitamin E, vitamin K, boron, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, selenium, zinc and bioflavonoid. For women up to 40 years of age, iron is included. For women over 40 years of age, iron is optionally included. The Ca/Mg ratio is in a range of 10-15/4-6.

[0031] A dietary supplement consisting of an extensive list of minerals and vitamins was described in U.S. Patent 5,654,011 (30). The patent sets forth no quantitative description on the contribution of each component to bone health.

SUMMARY OF THE INVENTION

[0032] The present invention describes formulations of a dietary supplement comprising acetate salts of calcium, magnesium, zinc and vitamin D_3. These preparations are highly soluble in water, gastric and intestinal fluids.

DETAILED DESCRIPTION OF THE FIGURES

[0033] **Figure 1** shows mean (±S.D.) percentage-time profiles of calcium of various formulas in artificial gastric juice (USP).

[0034] **Figure 2** shows the average cumulative net amount of calcium retained (±S.E.M.) in rats receiving calcium free diet over a four day period.

[0035] **Figure 3** shows the cumulative net amount of magnesium retained (±S.E.M.) in rats receiving calcium free diet over a four day period.

[0036] **Figure 4** shows the cumulative net amount of zinc retained (±S.E.M.) in rats receiving calcium free diet over a four day period.

[0037] **Figure 5** shows the plasma calcium (A), magnesium (B) and zinc (C) levels sampled from rats at the end of the treatment period while receiving calcium free diet.

[0038] **Figure 6** shows the average cumulative net amount of calcium retained (±S.E.M.) in rats receiving normal diet over a four day period.

[0039] **Figure 7** shows the average cumulative net amount of magnesium retained (±S.E.M.) in rats receiving normal diet over a four day period.

[0040] **Figure 8** shows the average cumulative net amount of zinc retained (±S.E.M.) in rats receiving normal diet over a four day period.

[0041] **Figure 9** shows the plasma calcium (A), magnesium (B) and zinc (C) levels sampled from rats at the end of the treatment period while receiving normal diet.

[0042] **Figure 10** shows the cumulative net amount of calcium retained (±S.E.M.) in rats receiving calcium free diet plus a daily consumed dose of calcium over a four day period.

[0043] **Figure 11** shows the cumulative net amount of magnesium retained (±S.E.M.) in rats receiving calcium free diet plus a daily consumed dose of calcium over a four day period.

[0044] **Figure 12** shows the cumulative net amount of zinc retained (±S.E.M.) in rats receiving calcium free diet plus a daily consumed dose of calcium over a four day period.

[0045] **Figure 13** shows the plasma calcium (A), magnesium (B) and zinc (C) levels sampled from rats at the end of the treatment period while receiving calcium free diet and a normal daily dose of calcium.

[0046] **Figure 14** is the body mass record of rats which received individual elemental treatments.

[0047] **Figure 15** shows trabecular BMD of Distal Femur Averaged from 3 pQCT Slices. *: significantly different from OVX-control ($p<0.05$).

[0048] **Figure 16** shows trabecular BMD of Proximal Tibia Averaged from 3 pQCT Slices. *: significantly different from OVX-control ($p<0.05$).

DETAILED DESCRIPTION OF THE INVENTION

[0049] In general, soluble calcium salts have a lower percentage of calcium. For example, calcium ascorbate has only 9% of calcium. The content is several folds lower than that of the insoluble calcium carbonate (40% calcium). Among soluble calcium, calcium acetate has the highest calcium content (25% calcium). This makes calcium acetate a suitable candidate for making a solid dosage.

[0050] The addition of elements and vitamins to a formula lowers the percentage of calcium. This poses a severe challenge to prepare a dosage form that has an acceptable size to consumers.

[0051] The present invention describes methodologies for preparing dosage forms with acceptable sizes.

[0052] The present invention also provides a method of preparing tablets comprising calcium acetate, magnesium acetate, zinc acetate and vitamin D_3, comprising the steps of: (i) blending a calcium composition comprising calcium acetate, magnesium acetate, and zinc acetate with a composition comprising vitamin D_3; and (ii) blending the composition obtained from (i) with a calcium composition comprising calcium acetate, magnesium acetate, and zinc acetate, thereby obtaining tablets comprising calcium acetate, magnesium acetate, zinc acetate and vitamin D_3. In one embodiment, the calcium composition comprises at least 10 percent by weight of calcium acetate, at least 5 percent by weight of magnesium acetate, and at least 0.2 percent by weight of zinc acetate.

[0053] The present invention also provides a tablet produced by the method described above.

[0054] The present invention also provides a method of preparing soft gel capsules comprising calcium acetate, magnesium acetate, zinc acetate and vitamin D_3, comprising the steps of: (i) dissolving vitamin D_3 in fish oil, flaxseed oil, or other oils containing either omega 3 or omega 3-6-9; (ii) mixing the composition obtained from (i) with a calcium composition comprising calcium

acetate, magnesium acetate, and zinc acetate, thereby obtaining soft gel capsules comprising calcium acetate, magnesium acetate, zinc acetate. In one embodiment, the calcium composition comprises at least 10 percent by weight of calcium acetate, at least 5 percent by weight of magnesium acetate, and at least 0.2 percent by weight of zinc acetate.

[0055] The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

[0056] Throughout this application, various references or publications are cited. Disclosures of these references or publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. It is to be noted that the transitional term "comprising", which is synonymous with "including", "containing" or "characterized by", is inclusive or open-ended and does not exclude additional, un-recited elements or method steps.

EXAMPLE 1
Formulations of Pearl Extracts

[0057] A pearl extract was prepared by adapting the patented method reported by Li and Li (31). Briefly, pearls are pulverized to a size between 80 to 120 mesh. The powder is soaked in a mixture of saturated sodium chloride solution with titrated amount of acetic acid. Electrical current is applied to the mixture for several days. After dilution with water and magnetization, the mixture was filtered and precipitated. The precipitate, rich in calcium acetate, is dried and ready for consumption as a dietary supplement. A detailed list of elements present in the extract is presented on Table 1:

TABLE 1
Content of Pearl Extract

Element	Quantity, ppm
Calcium	233,000
Magnesium	253
Zinc	3281
Potassium	1650
Manganese	1170

Sodium	680
Strontium	158
Molybdenum	55.4
Silicon	38.0
Selenium	27.9

[0058] This extract, A1, is fortified with acetate salts of magnesium to provide Ca/Mg ratios of 0.5/1 (A6), 1/1 (A4) and 2/1 (A5). The major elemental content of the pearl extract and its fortified mixtures are listed on Table 2:

TABLE 2
The Content of Each Element in Each Formula (n=3)

Formula No.	The content of three elements in each formula					
	Ca (%)		Mg (%)		Zn (%)	
	Determined	Labeled content[a]	Determined	Labeled content[a]	Determined	Labeled content[a]
A1	23.30±1.26	23.4	0.0253±0.0013	0.0012***	0.328±0.03	0.330
A4	7.65±0.62	7.51	7.56±0.32	7.50	0.372±0.029	0.375
A5	11.5±0.34	11.3	5.41±0.04	5.64	0.556±0.044	0.565
A6	4.58±0.09	4.50	8.29±0.15	8.99	0.256±0.012	0.225

Data are expressed as mean±S.D.
[a]In-house Data. ***p<0.001

[0059] Besides Pearl, the method described in this example can also be used to extract multiple acetate salts of calcium, magnesium and zinc from natural sources such as corals, oysters, mineral mines, etc. The composition of formulas A1, A4 through A6 could also be achieved by mixing appropriate amounts of acetates salts of calcium, magnesium and zinc.

[0060] Experimental Data on Elemental Solubility. The gastrointestinal tract is a complex organ. There are a number of factors which could alter the solubility of elements including calcium, magnesium and zinc; subsequently, their rate of absorption and bioavailability. Examples 2-5 highlight some of the physiological factors which have been postulated to have a significant impact on the solubility of elements.

EXAMPLE 2
Solubility of Calcium In Artificial Gastric and Intestinal Juice

[0061] The solubility of calcium in the four formulas in an artificial gastric (pH = 1) and intestinal fluid (pH =7) was tested using a method developed for ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (PerkinElmer Optima 4300DV). Two commercial samples, Caltrate™ and calcium acetate, were also tested in parallel for comparison. The results are shown in Table 3.

[0062] Compared to Caltrate™, the solubility of calcium acetate is approximately 45 times higher in the artificial gastric juice and 26,000 times higher in the artificial intestinal juice. The solubility of the pearl extract, A1, comprising mostly calcium acetate, is similar to that of calcium acetate in the artificial gastric juice and intestinal juice ($p>0.05$). The solubility of calcium acetate is pH dependent; it is lower in the artificial intestinal fluid when compared to the artificial gastric juice. Magnesium has a tendency to lower the solubility of calcium. When the ratio of Ca/Mg decreases, the solubility of the extract decreases, A5 > A4 > A6. Nevertheless, A6, the least soluble pearl extract formula, is ~12 times more soluble in artificial gastric juice and 8,500 times more soluble in artificial intestinal juice than that of Caltrate™. Therefore, unlike Caltrate™, solubility of acetate salts should not be an issue in gastrointestinal tract fluids because the acetate salts will still be in solution.

[0063] The solubility profile of magnesium salts is very similar to that of calcium (Table 4). In general, acetate salts of magnesium are highly soluble. They are more soluble in artificial gastric juice than artificial intestinal juice. In contrast to magnesium acetate, the solubility of magnesium carbonate in Caltrate™ is low.

[0064] The solubility profile of zinc salts is also similar to that of magnesium and calcium, except the magnitude of difference between salt forms under differing pH and environmental conditions is less drastic (Table 5).

[0065] This set of experiments thus leads to the conclusion that acetate salts are preferred salts in the disclosed formulations for their high solubility.

TABLE 3

Saturated Solubility of Calcium in Artificial Gastric And Intestinal Fluid (n=3)

	Saturated solubility of calcium	
Formula No.	Gastric fluid (g/L)	Intestinal fluid (g/L)
A1	72.93±4.14	64.97±6.29
A4	33.60±1.18	29.90±2.14

A5	53.97±8.34	45.50±7.24
A6	19.87±3.11	20.90±2.36
Calcium Acetate	77.73±8.13	68.43±2.55
Caltrate™	1.70±0.24	0.00246±0.00015

Data are expressed as Mean±S.D.

TABLE 4

Saturated Solubility Of Magnesium In Artificial Gastric Fluid And Intestinal Fluid

Formula No.	Saturated solubility of magnesium	
	Gastric fluid (g/L)	Intestinal fluid (g/L)
A1	0.13±0.006	0.13±0.04
A2	0.11±0.01	0.10±0.03
A3	0.12±0.04	0.09±0.01
A4	40.78±2.46	26.57±1.81***
A5	24.97±2.95	19.03±2.73***
A6	49.30±2.61	38.67±4.33***
Calcium Acetate	0.50±0.07	0.42±0.10
Caltrate™	0.17±0.17	0.09±0.02

Data are expressed as mean±S.D. (n=3).
***:P <0.001 compared with solubility in the artificial gastric fluid.

Table 5

Saturated Solubility Of Zinc In Artificial Gastric Fluid And Intestinal Fluid

Formula No.	Saturated solubility of zinc	
	Gastric fluid (g/L)	Intestinal fluid (g/L)
A1	1.04±0.16	0.76±0.07*
A2	3.72±0.68	2.14±0.14*
A3	3.25±0.19	2.31±0.08**
A4	2.22±0.17	1.19±0.11***
A5	2.64±0.38	1.64±0.07*
A6	1.54±0.13	1.07±0.11**
Calcium Acetate	0.60±0.17	0.53±0.14
Caltrate™	0.33±0.10	0.23±0.08

Data are expressed as mean±S.D. (n=3). *:P<0.05, **:P<0.01, ***:P<0.001 compared with the solubility in artificial gastric fluid.

EXAMPLE 3

Effects Of pH On The Solubility Of Calcium In Different Formulations

[0066] In this example, the effects of pH (ranging from 1 to 9) on the solubility of three elements of the four pearl formulas (A1, A4, A5 and A6), a commercial product (Caltrate™) and a synthetic compound (Calcium Acetate, Ca ACE) were investigated. Solution pH was adjusted using appropriate amounts of acetic acid (AcOH), nitric acid (HNO_3) or ammonium hydroxide (NH_4OH). Saturated solutions were prepared by dissolving each preparation in a solution with a final pH value

ranging from 1 to 9. The resultant mixtures were incubated in a water bath at 37°C for one hour. Each sample was then filtered (with or without centrifugation) immediately, and the filtrate was diluted to an appropriate concentration for elemental analysis. The concentration of calcium, magnesium, and zinc was measured using ICP-OES. The results are shown in Tables 6-8. Statistical analysis was performed using one-way ANOVA and the P value was set at 0.05.

[0067] Throughout the pH range tested, both A1 and calcium acetate showed significantly higher calcium content in solution than the other preparations. Caltrate™ had the lowest calcium content ($p<0.05$). A1 and calcium acetate have the highest solubility at pH 1 (Table 6)

[0068] Magnesium has a negative effect on the content of calcium in solution; the rank order in terms of solubility is A5>A4>A6. Except for Caltrate™, calcium acetate and A1, which are more soluble at pH 1, pH has no effect on the solubility of magnesium in solution (Table 7).

[0069] Similarly, the amount of zinc in solution correlated well with the zinc content in different formulations (A5>A4>A1>A6) (Table 8). For all four acetate formulas tested, pH values higher than 5 were associated with higher solubility of zinc than that at pH 2 and 3.

[0070] Since intestinal pH values are typically higher than 6, the present formulations present advantages in terms of solubility, when compared with the solubility of calcium carbonate in Caltrate™ under such pH conditions. These results are consistent with those reported in Table 3.

TABLE 6
Calcium Solubility (g/L) In Different pH Solutions (N = 3)

pH	Caltrate™	Ca ACE	A1	A4	A5	A6
1	4.60± 0.28	99.0 ± 19.2	101 ± 12.9	37.6 ± 2.5	48.7 ± 2.1	23.3 ± 3. 2
2	4.04 ± 0.23	61.3 ± 0.97	64.6± 5.1	29.4 ± 2.1	43.5 ± 5.3	22.6 ± 0.54
3	0.507 ± 0.10	75.7 ± 4.8	71.4 ± 22.2	38.0 ± 4.7	48.6 ± 0.98	19.5 ± 2.5
4	0.237 ± 0.03	85.4 ± 5.7	75.3 ± 3.4	38.5 ± 2.9	48.3 ± 0.82	20.8± 0.98
5	0.240 ± 0.06	75.6 ± 5.5	65.4 ± 11.8	39. 3 ± 4.4	47.4 ± 8.6	24.7± 2.5
6	0.317 ± 0.10	76.0 ± 5.9	83.8 ± 12.7	41.2 ± 1.3	52.3 ± 5.0	19.3 ± 2.7
7	0.133 ± 0.05	80.0 ± 3.5	84.2± 16.8	34.6 ± 3.3	49.5 ± 8.1	20.6 ± 3.8
8	0.160 ± 0.03	71.2 ± 1.6	78.3 ± 13.0	30.0 ± 3.8	55.3 ± 7.9	19.8 ± 2.0
9	0.227 ± 0.13	74.5± 6.8	84.8 ± 8.2	35.8 ± 3.5	50.2 ± 1.5	19.6 ± 4.2

TABLE 7
Magnesium Solubility (g/L) in Different pH Solutions (n = 3)

pH	Caltrate™	Ca ACE	A1	A4	A5	A6
1	0.197 ± 0.015	0.527 ± 0.121	0.173 ± 0.015	34.697 ± 4.836	23.927 ± 1.747	41.797 ± 5.622
2	0.100 ± 0.000	0.360 ± 0.010	0.133 ± 0.006	29.117 ± 2.204	20.020 ± 2.174	39.957 ± 1.050
3	0.133 ± 0.006	0.413 ± 0.035	0.143 ± 0.021	32.640 ± 41.65	21.880 ± 0.849	34.100 ± 5.169
4	0.123 ± 0.012	0.490 ± 0.010	0.173 ± 0.015	33.560 ± 2.606	21.733 ± 0.248	34.153 ± 1.560
5	0.107 ± 0.012	0.500 ± 0.040	0.137 ± 0.012	34.510 ± 2.817	24.367 ± 3.916	45.353 ± 7.294
6	0.110 ± 0.010	0.473 ± 0.076	0.177 ± 0.040	35.747 ± 1.738	24.997 ± 0.817	34.477 ± 4.730
7	0.093 ± 0.006	0.460 ± 0.035	0.153 ± 0.015	30.197 ± 2.818	21.677 ± 3.127	36.983 ± 7.234
8	0.097 ± 0.006	0.433 ± 0.040	0.157 ± 0.015	31.023 ± 6.548	24.953 ± 3.410	34.480 ± 4.046
9	0.097 ± 0.006	0.433 ± 0.045	0.160 ± 0.010	33.473 ± 7.169	23.607 ± 1.055	34.410 ± 6.836

TABLE 8

Zinc Solubility (g/L) in Different pH Solutions (n = 3)

pH	Caltrate™	Ca ACE	A1	A4	A5	A6
1	0.007 ± 0.006	0.030 ± 0.010	1.283 ± 0.220	1.980 ± 0.256	2.637 ± 0.143	1.440 ± 0.140
2	0.003 ± 0.006	0.013 ± 0.006	0.793 ± 0.093	1.457 ± 0.032	2.220 ± 0.204	1.143 ± 0.025
3	0.000 ± 0.000	0.017 ± 0.006	0.843 ± 0.315	1.593 ± 0.216	2.103 ± 0.134	0.817 ± 0.064
4	0.000 ± 0.000	0.017 ± 0.006	1.137 ± 0.092	1.867 ± 0.078	2.383 ± 0.071	0.933 ± 0.032
5	0.003 ± 0.006	0.017 ± 0.006	0.993 ± 0.195	1.930 ± 0.164	2.790 ± 0.305	1.250 ± 0.193
6	0.000 ± 0.000	0.020 ± 0.000	1.227 ± 0.133	2.063 ± 0.059	2.837 ± 0.135	0.870 ± 0.096
7	0.007 ± 0.012	0.023 ± 0.006	1.237 ± 0.223	1.770 ± 0.132	2.493 ± 0.372	0.990 ± 0.157
8	0.003 ± 0.006	0.027 ± 0.012	1.180 ± 0.180	1.787 ± 0.306	2.903 ± 0.300	0.940 ± 0.082
9	0.007 ± 0.006	0.027 ± 0.006	1.260 ± 0.087	1.970 ± 0.364	2.753 ± 0.133	0.917 ± 0.152

Experimental Data on Solubility in the Presence of Common Gastric and Intestinal Anions and Cations

[0071] The following analyses using anions which are present in abundance in gastro-intestinal tract fluids were performed on the four test formulas (A1, A4, A5 and A6), Caltrate™ and calcium acetate in order to assess the solubility and subsequently, their rate of absorption and bioavailability. The following are the standard ranges of common anions and cations in the human gastrointestinal fluids.

TABLE 9

Concentration Of Ions In Human Gastric And Intestinal Fluids

Ions	Concentration of ion (mM)	
	In Stomach / Gastric Fluid[a]	In Intestine / Intestinal Fluid[a]
Na^+	0 – 100 (0 – 80)	(155)
K^+	0 – 10 (0 – 15)	(70 – 150)
H^+	1 – 140 (20 – 120)	(pH 7.7 – 8.2)
Cl^-	100 – 170 (120 – 160)	(30 – 90)

Phosphate ions	Up to 100**
HCO_3^-	(70 – 130)

[a]Values were cited from *The Digestive System* (ISBN 0443062455). The values in brackets were cited from *The Medical Physiology* (ISBN 0781719364).
** based on the solubility of sodium phosphate.

EXAMPLE 4

Effects of Anions On The Solubility Of Calcium, Magnesium and Zinc In The Test Preparations

[0072] The following analyses using anions which are present in abundance in gastro-intestinal tract fluids were performed on the four test formulas (A1, A4, A5 and A6), Caltrate™ and calcium acetate in order to assess the solubility and subsequently, their rate of absorption and bioavailability. In this example, the effects of bicarbonate and phosphate (HCO_3^- and PO_4^{3-}) on the solubility of calcium, magnesium, and zinc were studied at pH 7. Furthermore, the effects of chloride on the absorption of these three elements at pH 1 and pH 7 were also studied. The procedures described in Example 3 for pH adjustment and solubility measurements were used. ICP-OES was used to quantify calcium, magnesium and zinc. Statistical analysis was performed using one-way ANOVA and the level of significance was set at $p<0.05$.

A. Chloride Effects at pH 1

[0073] Tables 10-12 are the results of chloride effects at pH 1. This condition mimics that of the acidic environment in the stomach. Chloride has the most intense effect on the solubility of calcium, magnesium and zinc in Caltrate™ at pH 1 (Tables 10-12). At a Cl^- concentration of 200 mM, the solubility of calcium was the highest. The maximum magnesium and zinc solubility was reached at Cl^- concentrations of 50 mM and 120 mM, respectively. The fluctuations of calcium, magnesium and zinc solubility are minimal in all the acetate formulations: calcium acetate, A1, A4, A5 and A6. Significant differences are often obtained at the highest Cl^- concentration ($p<0.05$).

TABLE 10
The Effect of Cl^- Concentration On The Solubility Of Calcium (g/L) In Different Formulations At pH 1

Cl^- Conc.	Caltrate™	Ca ACE	A1	A4	A5	A6
0 mM	4.597 ± 0.276	98.950 ±19.224	101.353 ±12.947	37.637 ± 2.509	48.670 ± 2.102	23.337 ± 3.162
50 mM	8.160 ± 0.497	80.857 ± 10.277	73.950 ± 0.987	29.950 ± 6.933	42.413 ± 12.931	22.290 ± 4.543
100 mM	7.333 ± 1.572	71.060 ± 1.660	85.627 ± 14.191	30.023 ± 4.042	43.853 ± 2.264	24.690 ± 0.746
120 mM	8.157 ± 1.210	76.453 ± 6.196	83.967 ± 0.479	36.883 ± 1.966	50.283 ± 2.977	24.850 ± 1.077
150 mM	5.883 ± 1.416	73.353 ± 1.037	87.340 ± 3.166	39.657 ± 4.659	44.443 ± 5.495	24.647 ± 0.775
180 mM	9.073 ± 0.325	80.977 ± 12.440	88.593 ± 5.579	41.710 ± 2.836	50.343 ± 1.392	26.067 ± 1.891
200 mM	12.123 ± 1.178	77.257 ± 12.364	97.840 ± 12.364	42.313 ± 6.119	63.027 ± 3.406	29.387 ± 4.062

TABLE 11
The Effect of Cl^- Concentration On The Solubility Of Magnesium (g/L) In Different Formulations At pH 1

Cl^- Conc.	Caltrate™	Ca ACE	A1	A4	A5	A6
0 mM	0.197 ± 0.015	0.527 ± 0.121	0.173 ± 0.015	34.697 ± 4.836	23.927 ± 1.747	41.797 ± 5.622
50 mM	0.357 ± 0.471	0.440 ± 0.075	0.133 ± 0.006	31.420 ± 6.649	20.547 ± 6.525	45.827 ± 6.006
100 mM	0.113 ± 0.012	0.380 ± 0.020	0.157 ± 0.012	35.853 ± 4.215	22.697 ± 1.231	46.900 ± 4.117
120 mM	0.243 ± 0.163	0.420 ± 0.036	0.140 ± 0.017	33.363 ± 2.542	23.333 ± 3.312	48.827 ± 4.095
150 mM	0.220 ± 0.132	0.403 ± 0.012	0.163 ± 0.015	36.037 ± 4.510	21.967 ± 1.260	45.653 ± 2.449
180 mM	0.227 ± 0.134	0.420 ± 0.040	0.160 ± 0.020	38.117 ± 3.356	24.210 ± 0.698	46.070 ± 3.290
200 mM	0.207 ± 0.074	0.427 ± 0.080	0.163 ± 0.006	43.203 ± 4.646	29.410 ± 0.115	81.437 ± 4.319

TABLE 12
The Effect of Cl^- Concentration On The Solubility Of Zinc (g/L) In Different Formulations At pH 1

Cl^- Conc.	Caltrate™	Ca ACE	A1	A4	A5	A6
0 mM	0.007 ± 0.006	0.030 ± 0.010	1.283 ± 0.220	1.980 ± 0.256	2.637 ± 0.143	1.440 ± 0.140
50 mM	0.030 ±0.000	0.027 ± 0.006	0.917 ± 0.156	1.500 ± 0.216	2.073 ± 0.598	1.237 ± 0.110
100 mM	0.130 ±0.026	0.067 ± 0.025	1.120 ± 0.010	1.683 ± 0.100	2.353 ± 0.057	1.293 ± 0.025
120 mM	0.217 ± 0.047	0.103 ± 0.031	1.113 ± 0.112	1.687 ± 0.196	2.487 ± 0.273	1.363 ± 0.095
150 mM	0.277 ± 0.091	0.073 ± 0.015	1.360 ± 0.144	1.803 ± 0.121	2.320 ± 0.106	1.280 ± 0.046
180 mM	0.180 ± 0.060	0.117 ± 0.031	1.193 ± 0.211	1.927 ± 0.015	2.590 ± 0.061	1.313 ± 0.032
200 mM	0.190 ± 0.056	0.123 ± 0.040	1.413 ± 0.187	2.230 ± 0.265	3.173 ± 0.248	2.083 ± 0.112

B. Chloride Effects at pH 7

[0074] At pH 7, the solubility of calcium in Caltrate™ is dramatically lower than that at pH 1 in the presence of chloride (Compare values in Tables 10 and 13). As chloride concentration increased, the solubility of calcium in Caltrate™ increased. The pH and chloride effects are not pronounced for the acetate formulations. In general, maximum calcium solubility is reached at chloride concentrations between 50 to 100 mM.

[0075] In the presence of chloride, pH has less of an effect on magnesium solubility (compare values between Tables 11 and 14). In general, the solubility of magnesium at pH 7 is slightly lower for all formulas and the chloride effect is not pronounced.

[0100] In the presence of chloride, the solubility of zinc in Caltrate™ at pH 7 is less than half of that at pH 1 (compare values between 11 and 14). However, this difference is not as pronounced in the acetate formulas. There is a tendency for zinc solubility to increase with the increase of chloride concentration. Maximum zinc solubility is reached at 120 mM chloride when Caltrate™ was evaluated. For the acetate formulas, maximum zinc solubility occurred when chloride concentration reached 200 mM.

TABLE 13
The Effect of Cl^- Concentration On The Solubility Of Calcium In Different Formulations At pH 7

Cl^- Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0 mM	0.133 ± 0.051	80.017 ± 3.505	84.170 ± 16.834	34.640 ± 3.268	49.497 ± 8.097	20.627 ± 3.821
50 mM	0.340 ± 0.082	99.373 ± 6.182	80.703 ± 13.103	47.473 ± 2.381	61.537 ± 6.436	31.490 ± 2.399
100 mM	0.557 ± 0.040	87.263 ± 13.984	77.660 ± 19.779	47.867 ± 7.511	66.743 ± 13.191	29.053 ± 6.684
120 mM	0.370 ± 0.165	71.440 ± 5.851	61.437 ± 8.616	35.400 ± 0.864	45.060 ± 6.166	22.353 ± 2.351
150 mM	0.567 ± 0.075	70.923 ± 3.240	73.773 ± 12.437	33.017 ± 2.455	42.980 ± 2.603	20.313 ± 2.005
180 mM	0.560 ± 0.165	77.823 ± 12.314	59.720 ± 7.467	34.003 ± 0.846	42.890 ± 5.516	17.490 ± 0.916
200 mM	0.600 ± 0.132	73.930 ± 7.785	84.707 ± 15.685	33.223 ± 2.093	46.403 ± 4.643	18.627 ± 2.238

TABLE 14
The Effect of Cl^- Concentration On The Solubility Of Magnesium In Different Formulations At pH 7

Cl^- Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0 mM	0.093 ± 0.006	0.460 ± 0.035	0.153 ± 0.015	30.197 ± 2.818	21.677 ± 3.127	36.983 ± 7.234
50 mM	0.280 ± 0.202	0.503 ± 0.031	0.140 ± 0.020	43.190 ± 2.792	29.203 ± 1.107	56.003 ± 3.989
100 mM	0.280 ± 0.149	0.480 ± 0.017	0.143 ± 0.006	45.253 ± 6.350	30.917 ± 6.111	52.953 ± 14.721
120 mM	0.110 ± 0.026	0.390 ± 0.030	0.147 ± 0.012	31.983 ± 3.302	19.333 ± 2.217	42.463 ± 1.448
150 mM	0.227 ± 0.096	1.750 ± 2.382	0.167 ± 0.015	29.087 ± 0.957	19.383 ± 1.482	42.643 ± 0.446
180 mM	0.253 ± 0.129	0.430 ± 0.046	0.167 ± 0.006	32.633 ± 2.372	19.733 ± 2.149	36.160 ± 10.009
200 mM	0.283 ± 0.107	0.427 ± 0.065	0.203 ± 0.025	32.923 ± 0.802	23.067 ± 2.175	47.133 ± 1.598

TABLE 15
The Effect of Cl^- Concentration On The Solubility Of Zinc In Different Formulations At pH 7

Cl^- Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0 mM	0.007 ± 0.012	0.023 ± 0.006	1.237 ± 0.223	1.770 ± 0.132	2.493 ± 0.372	0.990 ± 0.157
50 mM	0.113 ± 0.006	0.180 ± 0.089	0.997 ± 0.195	2.057 ± 0.189	3.177 ± 0.289	1.457 ± 0.244
100 mM	0.140 ± 0.026	0.213 ± 0.102	0.903 ± 0.280	2.413 ± 0.144	3.063 ± 0.287	1.540 ± 0.380
120 mM	0.050 ± 0.017	0.167 ± 0.202	0.760 ± 0.118	1.573 ± 0.146	1.997 ± 0.254	1.110 ± 0.036
150 mM	0.087 ± 0.025	0.177 ± 0.085	0.987 ± 0.110	2.030 ± 0.615	2.010 ± 0.165	1.177 ± 0.072
180 mM	0.093 ± 0.015	0.143 ± 0.071	0.780 ± 0.151	1.637 ± 0.127	2.090 ± 0.167	1.077 ± 0.163
200 mM	0.093 ± 0.012	0.160 ± 0.079	1.117 ± 0.202	1.663 ± 0.078	1.643 ± 1.217	1.303 ± 0.060

C. Bicarbonate Effects at pH 7.

[0101] The solubility of calcium in Caltrate™ increased with the increase of bicarbonate concentration (Table 16). However, the opposite is true for calcium acetate. The solubility was reduced at least 40%. The reduction for all the pearl extract formulas was less, approximately 20 to 25%.

[0102] The solubility of magnesium in Caltrate™ increased with bicarbonate concentration (Table 17). Bicarbonate effect was minimal for the acetate formulas.

[0103] The solubility of zinc in Caltrate™ increased in the presence of bicarbonate (Table 18). Maximum zinc solubility was reached at 70 mM. For calcium acetate, the trend is similar to that of Caltrate™. Bicarbonate has very little effect on the pearl extract formulas.

TABLE 16
The Effect of HCO_3^- Concentration On The Solubility Of Calcium In Different Formulations At pH 7

HCO_3^- Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0 mM	0.133 ± 0.051	80.017 ± 3.505	84.170 ± 16.834	34.640 ± 3.268	49.497 ± 8.097	20.627 ± 3.821
50 mM	0.217 ± 0.214	50.243 ± 3.312	72.030 ± 7.103	36.007 ± 3.807	42.577 ± 0.779	21.737 ± 1.255
70 mM	0.213 ± 0.098	62.090 ± 8.524	70.933 ± 4.812	33.420 ± 5.263	42.130 ± 4.734	22.343 ± 0.847
100 mM	0.380 ± 0.075	56.367 ± 9.062	83.640 ± 10.870	34.997 ± 6.049	46.167 ± 4.546	25.260 ± 10.191
120 mM	0.440 ± 0.167	46.023 ± 2.463	67.010 ± 3.767	31.060 ± 2.23	46.973 ± 2.919	20.817 ± 1.664
150 mM	0.433 ± 0.120	70.637 ± 3.622	65.617 ± 1.475	30.410 ± 2.888	41.567 ± 4.620	19.163 ± 1.568
180 mM	0.930 ± 1.290	46.847 ± 2.741	65.270 ± 1.781	28.680 ± 1.362	38.073 ± 3.465	18.870 ± 1.679

TABLE 17
The Effect of HCO_3^- Concentration On The Solubility Of Magnesium In Different Formulations At pH 7

HCO_3^- Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0 mM	0.093 ± 0.006	0.460 ± 0.035	0.153 ± 0.015	30.197 ± 2.818	21.677 ± 3.127	36.983 ± 7.234
50 mM	0.090 ± 0.035	0.297 ± 0.055	0.190 ± 0.056	34.600 ± 4.638	20.427 ± 1.272	48.140 ± 1.653
70 mM	0.093 ± 0.012	0.347 ± 0.031	0.160 ± 0.000	32.057 ± 4.407	22.000 ± 0.141	42.767 ± 0.460
100 mM	0.223 ± 0.111	0.343 ± 0.101	0.167 ± 0.065	41.580 ± 12.984	26.393 ± 4.720	43.883 ± 1.288
120 mM	0.220 ± 0.069	0.303 ± 0.015	0.483 ± 0.551	30.960 ± 2.164	22.877 ± 1.082	46.990 ± 5.278
150 mM	0.227 ± 0.072	0.410 ± 0.061	0.150 ± 0.017	28.950 ±2.262	18.850 ± 2.169	42.877 ± 7.608
180 mM	0.240 ± 0.095	0.293 ± 0.049	0.163 ± 0.015	30.787 ± 1.021	19.607 ± 1.529	36.957 ± 0.839

TABLE 18
The Effect of HCO_3^- Concentration On The Solubility Of Zinc In Different Formulations At pH 7

HCO_3^- Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0 mM	0.007 ± 0.012	0.023 ± 0.006	1.237 ± 0.223	1.770 ± 0.132	2.493 ± 0.372	0.990 ± 0.157
50 mM	0.057 ± 0.015	0.050 ± 0.010	0.953 ± 0.101	1.663 ± 0.205	2.010 ± 0.142	1.260 ± 0.017
70 mM	0.070 ± 0.020	0.100 ± 0.061	0.990 ± 0.082	1.560 ± 0.236	2.237 ± 0.099	1.147 ± 0.081
100 mM	0.070 ± 0.017	0.157 ± 0.055	1.190 ± 0.101	2.067 ± 0.654	2.660 ± 0.442	1.193 ± 0.023
120 mM	0.093 ± 0.025	0.210 ± 0.096	0.907 ± 0.042	1.513 ± 0.127	2.290 ± 0.115	1.317 ± 0.182
150 mM	0.087 ± 0.021	0.137 ± 0.083	0.863 ± 0.081	1.427 ± 0.059	1.887 ± 0.144	1.237 ± 0.235
180 mM	0.070 ± 0.017	0.160 ± 0.078	0.933 ± 0.072	1.517 ± 0.119	1.997 ± 0.157	1.023 ± 0.042

D. Effects of Phosphates at pH 7

[0104] Phosphates have insignificant effects on the solubility of calcium in Caltrate™ (Table 19). As phosphate concentrations increased the solubility of calcium decreased in all acetate formulations. Maximum reduction (up to 40%) of the solubility of calcium was observed in formulas containing higher percentage of magnesium (A4, A5 and A6). Considering the range of phosphate concentration tested, 10,000-fold, the change of calcium solubility is not significant.

[0105] Magnesium solubility decreased as phosphate concentration increased (Table 20). The reduction (80%) is most significant for the magnesium in Caltrate™. For the other formulas, the maximum reduction was approximately 50%. Again, the effect of phosphates was not that significant considering the range of concentration tested.

[0106] Among the three elements, phosphates have the most intense effect on the solubility of zinc (Table 21). All formulas were affected to the same extent and the maximum reduction was approximately 70%. Considering the range of phosphate concentration tested, again, the effects of phosphates were not that significant.

TABLE 19

The Effect of PO_4^{3-} Concentration On The Solubility Of Calcium In Different Formulations At pH 7

PO_4^{3-} Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0.01 mM	0.587 ± 0.200	77.517 ± 6.084	84.270 ± 9.511	34.950 ± 6.725	47.823 ± 3.080	22.287 ± 2.539
1 mM	0.510 ± 0.252	68.220 ± 19.638	56.450 ± 9.879	39.923 ± 10.060	42.363 ± 3.572	23.530 ± 0.159
10 mM	0.430 ± 0.046	78.417 ± 7.046	64.697 ± 9.058	25.703 ± 7.033	41.287 ± 3.584	21.687 ± 1.156
100 mM	0.453 ± 0.158	64.770 ± 1.548	58.607 ± 9.415	25.090 ± 3.181	34.650 ± 6.972	15.437 ± 2.428

TABLE 20

The Effect of PO_4^{3-} Concentration On The Solubility Of Magnesium In Different Formulations At pH 7

PO_4^{3-} Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0.01 mM	0.280 ± 0.070	0.493 ± 0.025	0.203 ± 0.006	38.017 ± 2.532	24.733 ± 0.886	52.000 ± 5647
1 mM	0.317 ± 0.087	0.450 ± 0.095	0.217 ± 0.031	35.647 ± 10.790	18.583 ± 1.676	48.967 ± 1.486
10 mM	0.240 ± 0.050	0.477 ± 0.035	0.173 ± 0.012	20.837 ± 5.545	18.163 ± 1.368	37.140 ± 2.681
100 mM	0.073 ± 0.006	0.350 ± 0.017	0.127 ±0.012	21.490 ± 1.830	16.720 ± 4.514	31.163 ± 4.838

TABLE 21

The Effect of PO_4^{3-} Concentration On The Solubility Of Zinc In Different Formulations At pH 7

PO_4^{3-} Conc.	Caltrate™ [g/L]	Ca ACE [g/L]	A1 [g/L]	A4 [g/L]	A5 [g/L]	A6 [g/L]
0.01 mM	0.117 ± 0.042	0.190 ± 0.070	1.193 ± 0.097	1.950 ± 0.040	2.750 ± 0.135	1.470 ± 0.154
1 mM	0.100 ± 0.044	0.197 ± 0.110	0.780 ± 0.151	1.800 ± 0.394	1.993 ± 0.093	1.380 ± 0.079
10 mM	0.070 ± 0.010	0.180 ± 0.089	0.767 ± 0.137	0.937 ± 0.253	1.740 ± 0.173	1.023 ± 0.060
100 mM	0.033 ± 0.015	0.053 ± 0.023	0.527 ± 0.119	0.623 ± 0.087	1.013 ± 0.345	0.510 ± 0.131

EXAMPLE 5

Effects of Cations On The Solubility Of Calcium, Magnesium and Zinc In The Test Preparations

[0107] The following analyses using cations which are present in abundance in gastro-intestinal tract fluids were performed on the four test formulas (A1, A4, A5 and A6), Caltrate™ and calcium acetate in order to assess the solubility and subsequently, their rate of absorption and bioavailability.

A. Effects of Na^+ at pH 1

[0108] The effects of Na^+ concentration on the solubility of the three elements in the four formulations (A1, A4, A5, and A6), Caltrate™ and CaACE were investigated at gastric pH (pH=1) and intestinal pH (pH=7), respectively. Tables 22 and 23 show the results tested at pH 1. No significant effects of Na^+ concentration on calcium and magnesium solubility of all formulations were observed. Solubility of zinc in Caltrate™ and calcium acetate, which contained trace amounts of Zn, increased significantly with an increase in sodium concentrations; however, no significant differences were obtained for all the acetate formulations (Table 24).

TABLE 22
Effect Of Concentration Of Na^+ On The Solubility Of Calcium Of Each Formula At pH 1

Na+ Conc.(mM)	Solubility of calcium (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	4.597 ± 0.276	98.950 ±19.224	101.353 ±12.947	37.637 ± 2.509	48.670 ± 2.102	23.337 ± 3.162
5	5.447 ± 2.061	84.600 ± 13.912	72.233 ± 1.501	36.467 ± 5.173	46.100 ± 0.721	22.000 ± 1.323
10	4.340 ± 0.035	66.967 ± 17.377	80.000 ± 1.852	40.033 ± 4.623	49.833 ± 2.503	27.900 ± 3.736
50	4.640 ± 0.707	90.167 ± 9.343	83.467 ± 3.313	36.633 ± 1.877	49.033 ± 4.452	25.467 ± 0.231
80	5.530 ± 0.946	87.167 ± 3.630	83.067 ± 6.813	37.033 ± 1.069	55.733 ± 5.372	30.600 ± 1.709
100	5.360 ± 0.742	79.233 ± 15.964	84.900 ± 11.609	39.100 ± 5.696	48.733 ± 3.968	25.067 ± 0.153

Data are expressed as mean±S.D. No statistical differences in all Na^+ concentrations tested for all formulations tested.

Table 23
Effect Of Concentration Of Na^+ On The Solubility Of Magnesium Of Each Formula At pH 1

Na+ Conc.(mM)	Solubility of magnesium (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.197 ± 0.015	0.527 ± 0.121	0.173 ± 0.015	34.697 ± 4.836	23.927 ± 1.747	41.797 ± 5.622
5	0.223 ± 0.006	0.700 ± 0.183	0.283 ± 0.040	36.400 ± 4.854	24.467 ± 1.361	39.933 ± 1.343
10	1.037 ± 1.109	0.483 ± 0.115	0.317 ± 0.050	38.967 ± 5.745	23.900 ± 1.800	49.100 ± 3.305
50	0.807 ± 0.889	0.620 ± 0.115	0.237 ± 0.031	35.733 ± 1.909	22.667 ± 2.055	45.500 ± 2.211
80	1.087 ± 1.264	0.580 ± 0.061	0.960 ± 1.031	35.033 ± 3.625	27.767 ± 3.700	50.900 ± 7.375

100	0.577 ± 0.525	0.497 ± 0.025	0.223 ± 0.032	36.000 ± 5.629	21.267 ± 2.120	46.233 ± 1.401

Data are expressed as mean±S.D. No statistical differences in all Na^+ concentrations tested for all formulations tested.

TABLE 24

Effect Of Concentration Of Na^+ On The Solubility Of Zinc Of Each Formula At pH 1

Na+ Conc.(mM)	Solubility of zinc (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.007 ± 0.006	0.030 ± 0.010	1.283 ± 0.220	1.980 ± 0.256	2.637 ± 0.143	1.440 ± 0.140
5	0.087 ± 0.006	0.123 ± 0.006	0.660 ± 0.128	1.393 ± 0.316	2.180 ± 0.413	1.183 ± 0.121
10	0.173 ± 0.015	0.317 ± 0.106	0.883 ± 0.080	1.767 ± 0.280	2.080 ± 0.160	1.760 ± 0.617
50	0.240 ± 0.053	0.400 ± 0.139	1.023 ± 0.075	1.727 ± 0.060	2.250 ± 0.114	1.410 ± 0.125
80	0.210 ± 0.026	0.397 ± 0.163	0.907 ± 0.211	1.730 ± 0.479	2.613 ± 0.270	1.747 ± 0.015
100	0.223 ± 0.031	0.363 ± 0.095	0.947 ± 0.188	1.490 ± 0.105	2.207 ± 0.506	1.493 ± 0.630

Data are expressed as mean±S.D

B. Effects of Na^+ at pH 7

[0109] Tables 25-27 show the effects of sodium ion at pH 7. Na^+ has no significant effects on calcium, magnesium and zinc solubility in general. It is interesting to note that all three elements in Caltrate™ could be not detected in the presence of Na^+ at pH 7.

TABLE 25

Effect Of Concentration Of Na^+ On The Solubility Of Calcium Of Each Formula At pH 7

Na+ Conc.(mM)	Solubility of calcium (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.133 ± 0.051	98.950 ±19.224	101.353 ±12.947	37.637 ± 2.509	48.670 ± 2.102	23.337 ± 3.162
10	---	83.300 ±26.469	67.433 ± 4.460	37.433 ± 4.822	43.800 ± 4.703	39.367 ± 16.110
50	---	69.000 ± 1.015	99.333 ± 21.548	35.533 ± 0.814	48.367 ± 4.359	23.833 ± 2.219
100	---	71.467 ±10.891	71.433 ± 1.193	36.867 ± 3.139	46.267 ± 1.380	24.567 ± 4.104
140	---	83.067 ± 6.596	68.900 ± 7.400	32.300 ± 1.153	47.200 ± 6.023	25.633 ± 3.754
170	---	72.333 ±15.467	71.433 ± 0.551	37.567 ± 10.473	43.133 ± 4.876	25.867 ± 3.175

Data are expressed as mean±S.D.
No statistical differences in all Na^+ concentrations tested for all formulations tested.

TABLE 26

Effect Of Concentration Of Na^+ On The Solubility Of Magnesium Of Each Formula At pH 7

Na+ Conc.(mM)	Solubility of magnesium (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.093 ± 0.006	0.527 ± 0.121	0.173 ± 0.015	34.697 ± 4.836	23.927 ± 1.747	41.797 ± 5.622
10	---	0.740 ± 0.165	0.110 ± 0.010	35.300 ± 3.579	19.500 ± 1.769	75.167 ± 34.360
50	---	0.427 ± 0.081	0.193 ± 0.015	35.933 ± 5.139	23.000 ± 4.327	52.167 ± 4.852
100	---	0.510 ± 0.066	0.157 ± 0.006	33.267 ± 3.889	20.667 ± 0.493	45.867 ± 3.329
140	---	0.497 ± 0.099	0.157 ± 0.021	28.867 ± 2.255	20.567 ± 2.610	51.000 ± 6.963
170	---	0.530 ± 0.036	0.167 ± 0.021	45.633 ±11.097	21.600 ± 2.476	53.500 ± 3.650

Data are expressed as mean±S.D.

TABLE 27

Effect Of Concentration Of Na^+ On The Solubility Of Zinc Of Each Formula At pH 7

Na+ Conc.(mM)	solubility of zinc (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.007 ± 0.012	0.030 ± 0.010	1.283 ± 0.220	1.980 ± 0.256	2.637 ± 0.143	1.440 ± 0.140
10	---	0.213 ± 0.102	0.600 ± 0.040	1.453 ± 0.185	1.543 ± 0.215	2.337 ± 1.351
50	---	0.280 ± 0.118	0.963 ± 0.280	1.700 ± 0.779	2.317 ± 0.798	1.687 ± 0.466
100	---	0.293 ± 0.129	0.707 ± 0.107	1.243 ± 0.211	1.790 ± 0.087	1.667 ± 0.275
140	---	0.320 ± 0.165	0.690 ± 0.137	1.113 ± 0.144	1.770 ± 0.056	1.643 ± 0.402
170	---	0.223 ± 0.102	0.730 ± 0.079	2.230 ± 0.397	1.933 ± 0.838	1.577 ± 0.529

Data are expressed as mean±S.D.

C. Effects of K^+ at pH 1

[0110] There is a tendency for the solubility of calcium to increase with an increase in potassium ion concentration (Table 28). However, most of the differences are not statistically different ($p<0.05$). In A5, the calcium solubility increased by more than 50%; this difference is significant ($p<0.05$).

[0111] Magnesium solubility profiles for the acetate formulas show a similar trend (Table 29) to that of calcium. There is a three-fold increase in the magnesium solubility in Caltrate™, ($p<0.05$). However, the magnitude of increase in inconsequential when compared to that of A4, A5 and A6.

[0112] An increase in potassium is associated with an increase in zinc solubility for Caltrate™ and CaACE (Table 30). Potassium has insignificant effect on the solubility of zinc in the four formulas ($p>0.05$). Again, the magnitude of increase in zinc solubility is inconsequential when compared to A4, A5 and A6

TABLE 28

Effect Of Concentration Of K^+ On The Solubility Of Calcium Of Each Formula At pH 1

K^+ Conc.(mM)	Solubility of calcium (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	4.597 ± 0.276	98.950 ±19.224	101.353 ±12.947	37.637 ± 2.509	48.670 ± 2.102	23.337 ± 3.162
2	4.300 ± 0.403	78.933 ± 1.320	71.833 ± 9.338	34.033 ± 1.739	35.833 ± 5.314	24.067 ± 1.474
5	3.607 ± 0.540	71.033 ± 13.079	73.733 ± 3.412	36.967 ± 1.159	47.500 ± 5.272	23.500 ± 1.778
10	6.497 ± 3.381	158.333 ± 40.624	83.733 ± 14.093	40.467 ± 7.823	66.567 ± 21.033	30.867 ±10.262
15	6.877 ± 0.956	161.667 ± 46.918	92.167 ± 14.793	41.867 ± 7.019	63.333 ± 7.651	26.667 ± 0.473
20	3.567 ± 0.501	100.800 ± 3.811	103.333 ± 15.822	42.633 ± 4.674	103.567 ± 64.463	29.300 ± 3.751

Data are expressed as mean±S.D.

TABLE 29

Effect Of Concentration Of K^+ On The Solubility Of Magnesium Of Each Formula At pH 1

K^+ Conc.(mM)	Solubility of magnesium (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.197 ± 0.015	0.527 ± 0.121	0.173 ± 0.015	34.697 ± 4.836	23.927 ± 1.747	41.797 ± 5.622
2	0.223 ± 0.087	0.693 ± 0.283	0.203 ± 0.029	34.933 ± 1.716	21.633 ± 4.300	49.700 ± 1.249
5	0.490 ± 0.419	0.453 ± 0.112	0.170 ± 0.030	32.667 ± 2.542	23.433 ± 3.408	43.000 ± 2.406
10	0.703 ± 0.846	0.820 ± 0.193	0.270 ± 0.130	38.733 ± 5.552	30.067 ± 8.429	55.400 ± 18.187
15	0.730 ± 0.912	0.687 ± 0.215	0.327 ± 0.185	41.467 ± 8.617	31.067 ± 4.050	54.800 ± 3.897
20	0.660 ± 0.764	0.650 ± 0.020	0.883 ± 1.140	52.067 ± 2.859	55.733 ± 34.208	54.233 ± 14.632

Data are expressed as mean±S.D.

TABLE 30

Effect Of Concentration Of K^+ On The Solubility Of Zinc Of Each Formula At pH 1

K+ Conc.(mM)	Solubility of zinc (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.007 ± 0.006	0.030 ± 0.010	1.283 ± 0.220	1.980 ± 0.256	2.637 ± 0.143	1.440 ± 0.140

2	0.053 ± 0.015	0.077 ± 0.006	0.607 ± 0.108	1.377 ± 0.221	1.937 ± 0.591	1.360 ± 0.122
5	0.173 ± 0.035	0.240 ± 0.075	0.790 ± 0.147	1.297 ± 0.169	2.593 ± 0.821	1.143 ± 0.278
10	0.203 ± 0.058	0.357 ± 0.111	1.127 ± 0.142	1.630 ± 0.185	2.373 ± 0.658	1.627 ± 0.225
15	0.193 ± 0.023	1.307 ± 1.199	1.060 ± 0.600	1.953 ± 0.590	2.963 ± 0.309	1.630 ± 0.161
20	0.167 ± 0.015	0.293 ± 0.093	1.100 ± 0.140	2.500 ± 0.236	5.450 ± 3.159	2.540 ± 1.424

Data are expressed as mean±S.D.

C. K^+ Effects at pH 7

[0113] There was a tendency for the solubility of calcium to increase with an increase in potassium concentration, however, the difference is not significant, $p>0.05$ (Table 31). No calcium could be detected in preparations using Caltrate™.

[0114] Similar observations to that of calcium were obtained for the solubility of magnesium and zinc ($p>0.05$) in all formulas containing acetate salts (Tables 32-33). No measurable magnesium and zinc was reported for preparations using Caltrate™.

TABLE 31

Effect Of Concentration Of K^+ On The Solubility Of Calcium Of Each Formula At pH 7

K+ Conc.(mM)	The solubility of calcium (g/L)					
	Caltrate™	Ca ACE	A1	A4	A5	A6
0	0.133 ± 0.051	98.950 ±19.224	101.353 ±12.947	37.637 ± 2.509	48.670 ± 2.102	23.337 ± 3.162
10	---	144.000 ± 14.731	66.800 ± 1.539	32.100 ± 0.361	64.033 ± 8.892	17.100 ± 0.173
50	---	174.467 ± 79.146	68.533 ± 3.259	33.933 ± 2.515	64.867 ± 17.244	19.033 ± 3.630
100	---	156.333 ± 64.361	68.600 ± 5.356	30.500 ± 3.672	82.000 ± 35.508	20.667 ± 2.363
140	---	130.033 ± 32.461	60.400 ± 25.999	56.767 ± 32.771	68.400 ± 7.100	42.000 ± 18.340
170	---	134.567 ± 55.048	126.133 ± 72.997	68.433 ± 29.905	64.800 ± 26.352	30.900 ± 14.912

Data are expressed as mean±S.D.
No statistical differences in all K^+ concentrations tested for all formulations tested.

TABLE 32

Effect Of Concentration Of K^+ On The Solubility Of Magnesium Of Each Formula At pH 7

K+ Conc.(mM)	The solubility of magnesium (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6

0	0.093 ± 0.006	0.527 ± 0.121	0.173 ± 0.015	34.697 ± 4.836	23.927 ± 1.747	41.797 ± 5.622
10	---	0.767 ± 0.189	0.140 ± 0.010	32.033 ± 2.829	30.967 ± 2.136	46.800 ± 3.158
50	---	1.027 ± 0.587	0.347 ± 0.316	33.533 ± 2.084	31.867 ± 8.151	48.200 ± 1.253
100	---	0.807 ± 0.278	0.183 ± 0.047	34.067 ± 3.465	39.233 ± 16.350	54.000 ± 2.955
140	---	0.817 ± 0.303	0.160 ± 0.035	57.833 ± 34.279	32.833 ± 5.541	90.467 ± 42.518
170	---	0.760 ± 0.310	0.230 ± 0.062	64.200 ± 26.513	31.333 ± 12.507	61.900 ± 30.685

Data are expressed as mean±S.D. No statistical differences in all K^+ concentrations tested for all formulations tested.

TABLE 33

Effect Of Concentration Of K^+ On The Solubility Of Zinc Of Each Formula At pH 7

K^+ Conc.(mM)	The solubility of zinc (g/L)					
	Caltrate™	CaACE	A1	A4	A5	A6
0	0.007 ± 0.012	0.030 ± 0.010	1.283 ± 0.220	1.980 ± 0.256	2.637 ± 0.143	1.440 ± 0.140
10	---	0.293 ± 0.110	0.727 ± 0.064	1.173 ± 0.163	3.243 ± 0.725	1.090 ± 0.070
50	---	0.627 ± 0.437	1.140 ± 0.036	1.447 ± 0.135	3.127 ± 0.720	1.247 ± 0.045
100	---	0.257 ± 0.110	1.197 ± 0.068	1.587 ± 0.106	3.417 ± 1.252	1.460 ± 0.122
140	---	0.387 ± 0.186	0.827 ± 0.506	2.583 ± 0.755	2.747 ± 1.432	2.607 ± 1.301
170	---	0.287 ± 0.142	1.223 ± 0.541	2.437 ± 0.618	2.873 ± 0.771	1.720 ± 0.624

Data are expressed as mean±S.D.

EXAMPLE 6

In Vivo Evaluation of Calcium, Magnesium And Zinc Balance

[0115] The objectives of the balance studies were to evaluate the effects of dietary conditions and formulations on calcium, magnesium and zinc balance.

A. Dietary Conditions

[0116] Two diets, one with normal calcium and the other is calcium free, were used for the studies. The nutrient composition of the diets is listed on Table 34:

TABLE 34

Composition Of Normal And Calcium Free Diet

	Normal	Calcium Free
Protein, %	24.0	19.0
Fat, %	4.5 (ether extract)	10.0

	6.0 (acid hydrolysis)	
Cholesterol, ppm	101	48
Fiber, %	5.3	5.4
Carbohydrates, %	21.5 (starch) 0.2 (Glucose) 0.2 (Fructose) 3.4 (Sucrose) 0.6 (Lactose)	60.6
Potassium, %	1.20	0.62
Sodium, %	0.40	0.27
Chlorine, %	0.70	0.27
Calcium, %	0.95	0.0
Magnesium, %	0.25	0.07
Zinc, %	0.011	0.0031
Iron, ppm	290	60
Manganese, ppm	110	65
Copper, ppm	17	23.9
Vitamin K, ppm	3.2	10.4
Riboflavin, ppm	12	20.0
Pyridoxine, ppm	8.0	16.5

B. Materials and Methods

[0117] Male Sprague-Dawley rats (about 6-7 weeks), with an initial weight between 220g to 250g, were randomly divided into different treatment groups. All the rats were housed in individual metabolic cages in a temperature-controlled room. Each rat received free access to the normal diet (Table 34) before the experiment. Both normal and calcium free diets (Table 34) were used in this set of studies. De-ionized water was provided *ad libitum*. All the rats were weighed before treatment.

C. Treatments

[0118] Two sets of studies were performed: a normal diet and calcium free diet. In each study, there were seven treatment groups. Thirty five animals were randomly assigned to one of the treatment groups in which one of the following were administered: Caltrate™, Calcium Acetate (Ca ACE), A1, A4, A5, A4 plus vitamin D_3 and A5 plus vitamin D_3 (n = 5 per group). Rats participating in the normal diet study received normal diet *ad libitum* throughout. Rats participating in the group of calcium free diet received the calcium free food *ad libitum* starting five days before and throughout treatment. In both study groups, animals received one dose a day for five days. Amounts of calcium, magnesium and zinc in individual formulation and in each diet were determined using ICP-OES. Values of dosage and dietary intake were measured for the calculation of elemental balance. For rats that were fed the normal diet, average daily elemental intake of calcium, magnesium and zinc was 625, 155 and 10 mg/kg/day, respectively. Daily elemental dosages, similar to that of human's, are 53.14 mg/kg for calcium, 0.38 to 55 mg/kg/day

for magnesium and 0.017 to 2.5 mg/kg/day for zinc. Vitamin D_3, 1.06 µg/kg/day (42.512 IU/kg/day; 1 IU=0.025 µg), was added to each dosage preparation prior to administration. The vehicle for preparing each dose was de-ionized water. The concentration of calcium in all dosage preparations was 15.94 mg/mL. One mL of each preparation was administered by gavage. Body weight, elemental dosage and diet consumption were recorded daily.

D. Sample Collection, Handling and Analysis

[0119] Animals were housed individually in a metabolic cage five days before the study. Food consumption was evaluated daily. Urine and feces were collected daily for four days and the content of calcium, magnesium and zinc was determined. On Day 5, each animal received its treatment. These treatments were administered once a day for four days. After the last treatment, each animal was anesthetized shortly before peak elemental blood concentration was achieved. Blood was collected using a heparinized syringe via cardiac puncture. Immediately after blood collection, the animal was then sacrificed with an overdose of isoflourane. Each blood sample was centrifuged at 1900 rpm at room temperature; plasma was harvested and stored at -20 °C until analysis. Urine was measured daily; it was diluted with de-ionized water, filtered and an aliquot was stored at -20 °C until analysis. Daily fecal output was collected and lyophilized. Each sample was weighed and digested using a mixture of three volume of nitric acid and one volume of perchloric acid. For every gram of dried feces, 10 mL of acid mixture was added. Each sample was digested for three days. The volume of the digested sample was measured and an aliquot of the digest was stored at -20 °C until analysis. The content of calcium, magnesium and zinc in plasma, feces and urine were determined using ICP-OES.

[0120] Daily calcium balance was calculated using equation 1:

[0121] Ca Balance = total Ca intake (dose and dietary intake) – Ca excreted in urine- Ca excreted in feces (1)

[0122] While, percentage of Ca balance was determined using equation 2:

[0123] % Ca balance = Ca balance / (total Ca intake) × 100% (2)

[0124] Cumulated calcium balance and % cumulated net calcium balance were calculated using equations (1) and (2), except, the sum of daily intake and excretion was used for calculation. The balance for magnesium and zinc was also calculated using the concept of equations (1) and (2).

Cumulated elemental balance and % cumulated net elemental balance were calculated in a similar fashion as described above.

[0125] In general, urinary excretion accounted for less than 5% of fecal excretion. Therefore, fecal excretion practically determines the quantity of elemental balance.

E. Statistical Analysis

[0126] All results were analyzed using two-way ANOVA. $P<0.05$ was considered to be significantly different. The data are presented as mean ± S.D. and mean ± S.E.M. in tables and figures, respectively.

F. Results: Calcium free diet

[0127] Table 35 shows the body weight of rats during the study. Stools from study animals were soft and this observation could be related to low elemental intake. Insufficient elements from the diet and dosage may have also caused the lack of weight gain for this set of animals. There is a statistical difference ($p<0.05$) among the starting body weights of the study animals (Table 35). There is also a slight in decline in body weight during the treatment period; is not the difference significantly different. .

TABLE 35

Body Weight Of Rats In Each Treatment Group With Calcium Free Diet (n=5)

Treatment group	Body weight of rats (g)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Caltrate™	184.6 ± 7.7	178.6 ± 9.9	177.2 ± 8.8	179.2 ± 13.7	175.4 ± 14.2
Ca ACE	202.4 ± 9.3	194.8 ± 9.3$^{\$}$	196.8 ± 10.9	193.4 ± 11.9$^{\$}$	188.0 ± 12.2$^{\$}$
A1	190.4 ± 11.9	185.6 ± 14.0*	187.6 ± 10.9	186.6 ± 11.3$^{\$}$	182.8 ± 15.4*
A4	188.4 ± 12.9$^{\$+}$	184.2 ± 13.2$^{\$+}$	184.0 ± 12.7	182.4 ± 13.5$^{\$+}$	183.2 ± 14.0*
A5	187.8 ± 8.8$^{\&\#}$	184.0 ± 6.0$^{\$\&\#}$	184.2 ± 5.6$^{\$*\#}$	185.4 ± 6.0$^{\$*+\&\#}$	182.4 ± 9.2$^{*\#}$
A4 + Vit D	207.6 ± 11.9$^{\$*+\&}$	200.0 ± 5.2$^{\$+}$	198.6 ± 4.5$^{\$*+\&}$	204.2 ± 4.4$^{\$\&@}$	199.8 ± 6.4$^{\$+\&}$
A5 + Vit D	204.8 ± 14.4$^{\$*+\&\%}$	195.6 ± 8.3$^{\$+\%}$	196.4 ± 7.7$^{*+\&\%}$	201.0 ± 5.0$^{\$\&\%}$	196.8 ± 8.2$^{\$+}$

$:P<0.05, compared with Caltrate™; *:P<0.05, compared with Ca ACE; +:P<0.01, compared with A1; &:P<0.05, compared with A4; %:P<0.001, compared with A5; #:P<0.001, compared with A4 + Vit D; @:P<0.05, compared with A5 + Vit D.

[0128] The addition of magnesium and zinc to a formula promotes the retention of calcium. A1, a composition with miniscule amounts of magnesium and zinc, has a lower calcium retention (17%,

Table 36); whereas the retention of calcium is significantly higher when the ratio of Ca/Mg was increased to 2/1 (A5), the calcium retention is 49% (Table 36). A higher proportion of magnesium, such as that present in A4, does not produce more changes in calcium retention (49%, Table 36). With respect to the minimum amount of magnesium required to provide the highest calcium retention, it appears a 2/1 Ca/Mg ratio is optimal.

[0129] The addition of vitamin D_3 increases calcium retention significantly (Figure 2 and Table 36). Calcium retention increased to 62% when vitamin D_3 was added to A5 (Table 36). This value is more than five times higher than that of the Caltrate™ and CaACE groups.

TABLE 36

Cumulative Net Percentage Of Calcium In Rats Treated With Elemental Supplements While Receiving Calcium Free Diet (n=5 per group)

Treatment group	Cumulative net percentage of calcium (%)			
	Day 1	Day 2	Day 3	Day 4
Caltrate™	23.8 ± 15.9*	22.3 ± 16.8	-2.27 ± 40.0	0.734 ± 35.7
Ca ACE	-30.6 ± 51.3	-9.88 ± 26.5	4.88 ± 24.0	11.1 ± 20.0
A1	37.5 ± 18.7*	20.9 ± 15.5	20.8 ± 15.0	17.2 ± 12. 1
A4	40.9 ± 19.1*	48.6 ± 13. 7*	49.1 ± 10.2$*	49.1 ± 7.7$*
A5	36.4 ± 24. 1*	46.8 ± 19.5*	48.7 ± 18.4$*	48.6 ± 19.1$*
A4 + Vit D	46.6 ± 22.3*	50.3 ± 10.9*	47.9 ± 14.8$*	50.8 ± 11.2$*
A5 + Vit D	43.7 ± 19.2*	52.7 ± 11.8*	59.2 ± 7.6$*+	62.0 ± 5.2$*+

$:P<0.05, compared with Caltrate™; *:P<0.05, compared with Ca ACE; +:P<0.05, compared with A1; #:P< 0.05, compared with A4 + Vit D.

[0130] Magnesium appears to be required in order to maintain magnesium balance (i.e. to avoid magnesium depletion) (Table 37). Formulas (Caltrate™, CaACE and A1) that have miniscule amounts of magnesium caused a net loss of magnesium (Figure 3 and Table 37).

[0131] The addition of vitamin D_3 has no significant effect on the retention of magnesium. The cumulative net percentage of magnesium did not change significantly after vitamin D_3 was added to A4 and A5 (Figure 3 and Table 37).

TABLE 37

Cumulative Net Percentage Of Magnesium In Rats Treated With Elemental Supplements

While Receiving Calcium Free Diet (n=5 per group)

Treatment group	Cumulative net percentage of magnesium (%)			
	Day 1	Day 2	Day 3	Day 4
Caltrate™	-191.9 ± 139.1	-125.6 ± 51.0	-111.8 ± 39.1	-116.5 ± 37.7
Ca ACE	-197.2 ± 105.2	-150.4 ± 88.9	-115.3 ± 62.7	-93.6 ± 37.2
A1	-47.3 ± 22.4$^{\$*}$	-67.9 ± 33.3*	-55.2 ± 13.4	-64.4 ± 24.6
A4	66.5 ± 8.7$^{\$*+}$	68.1 ± 6.4$^{\$*+}$	65.8 ± 5.9$^{\$*+}$	60.9 ± 4.7$^{\$*+}$
A5	23.7 ± 46. 3$^{\$*}$	37.6 ± 37.1$^{\$*+}$	41.1 ± 34.2$^{\$*+}$	39.6 ± 33.3$^{\$*+}$
A4 + Vit D	46.3 ± 27.5$^{\$*+}$	49.3 ± 18.8$^{\$*+}$	49.3 ± 15.3$^{\$*++}$	48.9 ± 15.5$^{\$*+}$
A5 + Vit D	16.0 ± 20.0$^{\$*}$	23.9 ± 21.5$^{\$*+}$	28.9± 17.9$^{\$*+}$	27.2 ± 23.0$^{\$*+}$

$:P<0.05, compared with Caltrate™; *:P<0.05, compared with Ca ACE; +:P<0.05, compared with A1.

[0132] The retention of zinc is highly variable; it is particularly true with formulas such as Caltrate™, calcium acetate and A1 that contain minute amounts of zinc (Table 38). The results also show that zinc balance became negative when the amount of zinc is low.

[0133] The addition of zinc to formulas such as A4 and A5 did not significantly improve zinc balance (Table 38). The addition of magnesium to the formulas may have caused zinc balance to stay negative (Figure 4).

[0134] However, the addition of vitamin D_3 to A4 and A5 made zinc balance positive (Figure 4 and Table 38). The importance of vitamin D_3 on zinc is clearly demonstrated in this set of studies.

[0135] Figure 5 shows plasma elemental profiles after each treatment. There were no significant differences observed after elemental treatments.

TABLE 38

Cumulative Net Percentage Of Zinc In Rats Treated With Elemental Supplements While Receiving Calcium Free Diet (n=5 per group)

Treatment group	Cumulative net percentage of zinc			
	Day 1	Day 2	Day 3	Day 4
Caltrate™	-50. 6 ± 50.0	-38.7 ± 23.8	-36.9 ± 26.4	-39.5 ± 23.7
Ca ACE	-107.1 ± 85. 5	-77.7 ± 59.0	-65.7 ± 66.7	-50.5 ± 46.4

A1	10.1 ± 8.7[$&]	-0.348 ± 22.2[$&]	4.22 ± 7.3[$&]	-2.79 ± 6.4
A4	-61.0 ± 38.8[$]	-55.3 ± 29.3[$]	-58.3 ± 24.5[$]	-33.8 ± 23.9[$]
A5	-8.05 ± 45.3[$&]	9.737 ± 39.5[$*&]	9.96 ± 40.3[$*&]	8.76 ± 40.1[$*]
A4 + Vit D	27.2 ± 40.7[$*&]	43.7 ± 18.8[$*&]	51.2 ± 15.1[$*&]	54.2 ± 11. 2[*&]
A5 + Vit D	22.8 ± 17.9[*&]	35.8 ± 17.8[*&]	42.9 ± 12.9[*&]	44.6 ± 10.1[*&]

$:P<0.05, compared with Caltrate™; *:P<0.05, compared with Ca ACE;
&:P<0.05, compared with A4

G. Results: Normal diet

[0136] Rats that received normal diet gained weight (Table 39). Elemental treatments have no significant effect on weight gain ($p>0.05$).

TABLE 39
Body Weight Of Rats Receiving Normal Calcium Diet (n=5)

Treatment group	Body weight of rats (g)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Caltrate™	228.8 ± 4.6	232.8 ± 2.6	233.8 ± 3.5	243.6 ± 8.9	243.8 ± 5.1
Ca ACE	242.0 ± 7.4	237.0 ± 12.5	239.2± 13.9	238.6 ± 13.9	244.0 ± 12.8
A1	230.0± 4.5	233.8 ± 8.0	238.2 ± 6.1	244.6 ± 7.2	244.6 ± 3.5
A4	234.8 ± 7.7	238.6 ± 5.1	238.2 ± 5.9	239.0 ± 5.1	245.8 ± 4.9
A5	239.6 ± 10.3	243.0 ± 13.9	245.4 ± 13.6	245.4 ± 13.4	248.6 ± 14.4

Data are expressed as mean±S.D.

[0137] The pattern of calcium retention appears to be similar to that obtained from rats that received calcium free diet (compare Tables 36 and 40); suggesting calcium balance is dependent upon elemental treatments, despite the fact that the amount of calcium administered was approximately 10% of the animal's daily dietary intake (~130 to 140 mg of calcium per day). This observation strongly suggests that dietary calcium, present in the least absorbable carbonate form, was enhanced by elemental treatments. The treatment with Caltrate™ has minimal effect. It is not surprising because Caltrate™ contains only calcium carbonate. The treatment with A5 has the most pronounced effect (Figure 6 and Table 40).

TABLE 40
Cumulative Net Percentage Of Calcium In Rats Treated With Elemental Supplements

While Receiving Normal Diet (n=5 per group)

Treatment group	Cumulative net percentage of calcium (%)			
	Day 1	Day 2	Day 3	Day 4
Caltrate™	-6.9 ± 24.6	17.3 ± 7.5	21.3 ± 10.0	17.5 ± 10.2
Ca ACE	14.4± 24.0	26.9 ± 9.0	30.3 ± 4.9	31.9 ± 3.0
A1	31.4 ± 33.5$	49.2 ± 38.8$	39.2 ± 27.3	31.3 ± 21.9
A4	19.3 ± 12.6	23.7 ± 9.4	26.2 ± 9.6	22.7 ± 7.3
A5	52.1 ± 21.7$*&	49.0 ± 19.8$	48.9 ± 20.4	45.3 ± 22.7

$:P<0.05, compared with Caltrate™; *:P<0.05, compared with Ca ACE; &:P<0.05, compared with A4

[0138] Average dietary intake of magnesium by the study animals was approximately 35 mg. Magnesium balance for all study groups was positive (Figure 7 and Table 41). This observation is consistent with the observation obtained from animals receiving the calcium free diet, in that magnesium intake is required to maintain a positive balance (Tables 37 and 41). Interestingly, the day to day trend showed that animals treated with acetate formulas (CaACE, A1, A4 and A5 vs. Caltrate™) had consistently higher percentages of magnesium balance.

TABLE 41
Cumulative Net Percentage Of Magnesium In Rats Treated With Elemental Supplements While Receiving Normal Diet (n=5 per group)

Treatment group	Net accumulative percentage of magnesium (%)			
	Day 1	Day 2	Day 3	Day 4
Caltrate™	-2.82 ± 19.6%	23.3 ± 8.1%	27. 3 ± 10.0	24.6 ± 6.9
Ca ACE	16.7± 17.2%	29.9 ± 3.8	34.3 ± 2.5	37.7 ± 2.7
A1	11.7 ± 11.7%	44.1 ± 30.7	38.9± 22.9	31.5 ± 17.0
A4	28. 2 ± 9.1$	34.0 ± 7.8	36.8 ± 7.2	35.0 ± 4.4
A5	48.9 ± 25.3	48.9 ± 20.9	50.6 ± 20.1	48.6 ± 21.0

$:P<0.05, compared with Caltrate™; %P:<0.05, compared with A5

[0139] There were no statistical differences among elemental treatments in terms of zinc balance (Figure 8 and Table 42). The quantity of zinc administered via elemental formulas was no more than 30% of the daily dietary intake. It was noted that the addition of a high quantity of

magnesium tended to lower zinc balance, a trend observed with A4 treatment (Figure 8 and Table 42). This observation is similar to that observed in the calcium free diet study (Table 38).

[0140] Contrary to the calcium free diet study (Table 38), zinc balance was positive in this study (Table 42). This was achieved without vitamin D_3 (Figures 4 and 8, Tables 38 and 42). This apparent discrepancy may be due to the quantity of total zinc intake and/or the rate at which zinc was consumed. Elemental consumption, along with other nutrients, occurred throughout the feeding period which may last up to 12 hours; whereas elemental treatments were given as a bolus. Concentration and ratio of nutrients presented to the intestinal wall may have a huge difference between bolus administration and dietary consumption. These differences could account for the difference in zinc balance.

[0141] The results from the calcium free and normal diet studies clearly suggest that adequate dietary intake of elements is key to elemental balance. Elemental and vitamin D_3 supplementation are necessary if the diet in deficient in these nutrients.

[0142] Figure 9 shows plasma concentration of calcium, magnesium and zinc after individual elemental treatments. There were no statistical differences in the concentration of these elements in plasma after elemental treatments ($P>0.05$).

TABLE 42
Cumulative Net Percentage Of Zinc In Rats Treated With Elemental Supplements While Receiving Normal Diet (n=5 per group)

Treatment group	Cumulative net percentage of zinc (%)			
	Day 1	Day 2	Day 3	Day 4
Caltrate™	0.67 ± 34.7%	29.5± 7.5	33.8 ± 10.2	32.0 ± 7.8
Ca ACE	27.5 ± 16.0%	40.9 ± 7.3	45.6 ± 5.9	48.4 ± 4.4
A1	26.6 ± 11. 2%	50.8 ± 26.8	46.3 ± 20.4	38.7 ± 18.8
A4	17.7 ± 10.3%	24.9 ± 6.3%	27.6 ± 7.2	27.6 ± 5.0
A5	54.7 ± 21.9	52.6 ± 21.7	53.8 ± 21.0	51.2 ± 23.0

%:P<0.05, compared with A5

H. Results: Calcium Free Diet with Daily Consumed Doses of Calcium

[0143] The objective of this study was to evaluate elemental balance when the daily intake of calcium, magnesium and zinc was replaced with elemental treatments. Animals, received de-

ionized water *ad libitum* (DI Water group), were fed normal calcium diet. Animals, substituting their daily calcium intake by A1 or A5, were fed calcium free diet. It is apparent that the gavage procedure did not have an effect on the body weight of the animals (Table 43). Elemental treatments, however, induced a significant reduction in body weight.

TABLE 43
Body Weight Of Rats Receiving Calcium Free Diet And Daily Consumed Doses Of Calcium (n=4)

Treatment group	Body weight of rats (g)				
	Day 1	Day2	Day3	Day4	Day5
DI Water	200.8 ± 2.50	207.0 ± 3.9	209.0 ± 8.7	209.5 ± 9.9	215.5 ± 11.7
A1	198.0± 9.1	183.5 ± 7.7	178.3 ± 8.1	180.8 ± 10.2	186.0 ± 8.0
A5	194.0 ± 8.2	182.3 ± 7.1	179.8 ± 7.2	179.0 ± 7.7	181.5 ± 6.8

Note: There is no statistical significant difference between A1 and A5. There is statistical difference between A1 and DI ($p < 0.001$), and between A5 and DI ($p<0.001$).

[0144] Contrary to the results obtained from the normal and calcium free diet studies, magnesium has a minor effect in enhancing calcium retention (Figure 10 and Table 44). The administration of a soluble form of calcium, calcium acetate, significantly enhanced calcium balance (Figure 10 and Table 44).

Table 44
Cumulative Net Percentage Of Calcium In Rats Treated With A Daily Consumed Dose Of Calcium While Receiving Calcium Free Diet (n=4 per group)

Treatment group	Net accumulative percentage of Ca (%)			
	Day 1	Day 2	Day 3	Day 4
DI Water	2.87 ± 5.4	3.89 ± 7.6	5.72 ± 4.3	5.41 ± 5.2
A1	46.3 ± 14.7*	37.7 ± 8.9*	37.4 ± 1.3*	42.7 ± 3.1*
A5	54.9 ± 12. 7*	56.7± 10.3*@	50.4 ± 7.5*	47.4 ± 8.0*

*:P<0.05, when compared with DI; @:P<0.05m when compared to A1

[0145] Consistent with the calcium free diet study described above, magnesium was required to maintain a positive magnesium balance (Figure 11 and Table 45).

Table 45

Cumulative Net Percentage Of Magnesium In Rats Treated With A Daily Consumed Dose Of Calcium While Receiving Calcium Free Diet (n=4 per group)

Treatment group	Net accumulative percentage of Mg (%)			
	Day 1	Day 2	Day 3	Day 4
DI Water	-32.2 ± 12.4	-17.0 ± 10.4	-7.9 ± 10.0	-2.59 ± 10.4
A1	-75.9 ± 50.0*	-27.6± 27.7	-6.54 ± 19.4	3.6 ± 18.0
A5	18.3 ± 12.7*@	14.4 ± 8.6@	7.0 ± 5.2	4.4 ± 8.4

*:P<0.05, when compared with DI; @:P<0.05m when compared to A1

[0146] Despite a higher amount of zinc administered with A5, zinc balance was significantly lower than that of the DI Water group, providing further support that high calcium and magnesium concentration in the intestine could have diminished zinc absorption. (Figure 12 and Table 46). The amounts of zinc administered between the DI Water and A1 groups were similar. However, similar to that of A5, zinc balance was significantly lower than that of DI Water (Figure 12 and Table 46); suggesting high solution concentration of calcium in the intestine may interfere with zinc absorption.

[0147] This set of results suggest that elemental dietary intake of elements does not produce the same effects when compared to that of an equivalent bolus dose.

[0148] Taking all the study results into consideration, A5 produces the most consistent calcium balance under different experimental/dietary conditions (compare results on Tables 36, 40 and 44). The addition of vitamin D_3 enhances calcium retention of A5 when the subject is deficient in dietary elements (Table 36).

[0149] Figure 13 shows plasma concentrations of calcium, magnesium and zinc after each elemental treatment. No statistical differences were found in these profiles (P>0.05).

TABLE 46

Cumulative Net Percentage Of Zinc In Rats Treated With A Daily Consumed Dose Of Calcium While Receiving Calcium Free Diet (n=4 per group)

Treatment group	Net accumulative percentage of Zn (%)			
	Day 1	Day 2	Day 3	Day 4

DI Water	-26.5 ± 37.7	-10.8 ± 22.9	-4.45 ± 17.3	-1.80 ± 12.2
A1	-42.9 ± 25.9	-67.3 ± 16.3*	-69.5 ± 7.3*	-58.5 ± 6.2*
A5	23.7 ± 16.2*@	-9.09 ± 19.3@	-45.1± 11.8*	-63.2 ± 16.2*

*:P<0.05, when compared with DI; @:P<0.05, when compared to A1

EXAMPLE 7

[0150] The objectives of this study were to evaluate the effects of salt, mineral composition and vitamins on the rate of bone loss in an ovariectomized rat model.

[0151] One hundred 4.5-month-old female Sprague-Dawley rats were used and housed at the Laboratory Animal Services Center at the Chinese University of Hong Kong with 12-h light-night cycle. Free cage movement was allowed with access to the normal calcium pellets and tap water. Daily consumption of calcium was approximately 140 mg, similar to that recorded in animals who participated in the balance studies. Ovariectomy (OVX), the removal of ovaries from the female rats, was performed on all rats at 6-month of age with the exception of the sham control.

[0152] Three weeks after OVX, all the rats recovered from the trauma of the surgery. The rats were randomly divided into different treatment groups or control groups and each group contained six rats. Four calcium formulas (A1, A4, A5 and A6) and Caltrate™ were investigated in the present study. The Caltrate™ group served as an elemental treatment control. All formulas were dissolved in distilled water, while Caltrate™ was in suspension in distilled water. The solution or suspension was given to the rats daily for 8 weeks by gavages. The dose of all formulas was calculated based on a calcium dose of 53.14 mg/kg/day. Dose of vitamin D_3 and vitamin K_2 was 12.75 IU/kg/day (equivalent to 800 IU/70 kg man/day) and 1.71 µg/kg/day (equivalent to 120 µg/ 70 kg man/day), respectively. All the treated rats were weighed daily and the mass data were recorded. The rats in two control groups (sham control and normal control) were given the equivalent volume of distilled water in parallel. For the groups with the treatment of bisphosphonate, alendronate (14 µg/kg/2-week) was injected subcutaneously on the back of the rats once every two weeks.

[0153] At the end of 8 weeks, the rats were anesthetized using isoflourane. Blood sample was then taken via heart puncture. The rats were then euthanized under anesthesia by neck dislocation, and right hip, right femur and right tibia of each rat were collected for analysis. Plasma was

collected from blood samples centrifuged at 1500 *g* for 15 min. Plasma concentrations of calcium, magnesium, and zinc were measured using ICP-OES.

[0154] Results show that plasma calcium levels were not statistically different from that of the sham control ($p>0.05$) and the values are all within normal levels (90-110 mg/L). All plasma concentrations of Mg were within the normal range (18–36 mg/L). No significant difference in magnesium plasma concentrations was observed except normal control (without surgery) has a mean value higher than that of A4+Vit D+Vit K ($p<0.05$). Similarly, plasma concentrations of Zn in all rats reached the rat normal concentration at about 1.26 mg/L. Zn plasma concentrations of rats in the normal control was significantly higher than that of sham control rats and also the rats treated with A5+vitamin D and A4+vitamine D+vitamin K ($p<0.05$).

[0155] Body weight changes for different treatment groups are shown in Figure 14. As expected, weight gains in the OVX rats were significantly greater than the normal rats ($p<0.05$).

[0156] The effects of test substances on bone mineral density (BMD) are shown on Figures 15 and 16. Trabecular BMD of Distal Femur BMD values of groups A1, A5+Vit D, Bis+A1+Vit D, Bis+A4+Vit D, Bis+A5+Vit D and Bis+ Caltrate +Vit D are significantly higher than that of the OVX control (Figure 15), suggesting these treatments significantly slow down the rate of loss of bone mass. The addition of vitamin K did not have any significant effect on reducing the rate of bone loss. Similar observations were obtained for the average values of trabecular BMD of Proximal Tibia, except the value of Caltrate™ was high enough to become statistically different ($p<0.05$, Figure 16). Again, vitamin K did not have any significant contribution. The treatment with A5+Vit D provided consistently higher BMD at distal femur and proximal tibia, suggesting this formula may have an advantage over the other elemental formulas. Although, the addition of bisphosphonate provides consistently better results, the difference, when compared to A5+Vit D and other elemental formula, such as A1, was not significant (Figures 15 and 16).

[0157] The BMD results of A1 are similar to that of A5 + vit D. This is not surprising because A1 animals were fed normal calcium diet which contains a significant amount of magnesium.

[0158] The OVX rat model used in this study did not permit evaluation of maximum bending force and failure energy after each treatment because the values obtained from the OVX control and that of the Sham were insignificantly different from each other ($P>0.05$).

EXAMPLE 8
Fortification of Juices with A5

[0159] Fruit juices contain a number of acids such as malic acid, citric acid, etc. which may alter the solubility and hence the recovery of the three key elements in the formulae, hence changing the absorbability of these elements when administered in juice format.

[0160] The objectives of this study were to evaluate the effects of temperature and storage on the recovery of calcium, magnesium and zinc in A5 after mixing with filtered and unfiltered orange, grape and carrot juice.

[0161] A 2.6 g or 500 mg amount of A5 was weighed accurately and mixed with 330 ml of water or either filtered or unfiltered grape, orange or carrot juice. The specimens were prepared at either 4 or 21 °C. The elemental content was measured using ICP-OES.

[0162] Small quantities of calcium, magnesium and zinc were found in orange, grape and carrot juice (Tables 47, 50 and 53). Temperature and filtration had no effects on the recovery of calcium, magnesium and zinc of A5 when 2.6 g of A5 was used for the study (Tables 48, 51 and 54).

TABLE 47

Content of the three key elements in fresh orange juice

Sample	Content (mg/L)		
	Ca	Mg	Zn
Fresh orange juice	87.7 ± 0.87	115 ± 0.9	0.47 ± 0.03

Data are expressed as Mean ± S.D. (n=3)

TABLE 48

Elemental recovery of the 3 key elements of A5 (2.6 g) in orange juice at 4°C and 21°C

Sample	Solubility (g/L)					
	Ca		Mg		Zn	
	4°C	21°C	4°C	21°C	4°C	21°C
Unfiltered	0.968 ± 0.006	0.997 ± 0.002	0.528 ± 0.007	0.542 ± 0.012	0.047 ± 0.004	0.045 ± 0.000
Filtered	0.978 ± 0.008	0.994 ± 0.008	0.521 ± 0.007	0.527 ± 0.002	0.045 ± 0.001	0.045 ± 0.003

Data are expressed as mean ± S.D. (n=3)

TABLE 49

Elemental recovery from 500 mg of A5 in 330 ml orange juice stored at 4 °C

	Solubility (g/L)					
	Ca		Mg		Zn	
	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered
Fresh	0.273 ± 0.005	0.274 ± 0.009	0.170 ± 0.001	0.167 ± 0.003	0.0105 ± 0.0012	0.0089 ± 0.0003
One Week	0.272 ± 0.005	0.172 ± 0.017***	0.171 ± 0.001	0.104 ± 0.010***	0.0104 ± 0.0015	0.0139 ± 0.0088

Data are expressed as Mean ± S.D. (n=3) ***P<0.001 comparing with fresh group

TABLE 50

Content of the three key elements in fresh grapefruit juice

Sample	Content (mg/L)		
	Ca	Mg	Zn
Fresh grapefruit juice	48.2 ± 0.79	104 ± 1.6	0.536 ± 0.008

Data are expressed as mean ± S.D. (n=3)

TABLE 51

Comparison of elemental recovery of A5 (2.6 g) in grapefruit juice at 4°C and 21°C

Sample	Solubility (g/L)					
	Ca		Mg		Zn	
	4°C	21°C	4°C	21°C	4°C	21°C
Unfiltered	0.958 ± 0.010	0.968 ± 0.016	0.55 ± 0.005	0.518 ± 0.010	0.046 ± 0.001	0.046 ± 0.001
Filtered	0.981 ± 0.018	0.975 ± 0.004	0.516 ± 0.027	0.520 ± 0.005	0.045 ± 0.002	0.048 ± 0.002

Data are expressed as mean ± S.D. (n=3)

TABLE 52

Elemental recovery from A5 (2.6 g) in distilled water at 4 and 21 °C

Temperature	Solubility (g/L)		
	Ca	Mg	Zn
4 °C	0.875 ± 0.018	0.407 ± 0.000	0.024 ± 0.002
21 °C	0.897 ± 0.016	0.404 ± 0.009	0.028 ± 0.001

Data are expressed as Mean ± S.D. (n=3)

[0163] Similarly, temperature has no effect on the recovery of A5 elements in distilled water (Table 52).

[0164] Storage at 4 °C for a week did not change the recovery of calcium, magnesium and zinc when 2.6 g of A5 was dissolved in 330 ml of filtered and unfiltered orange and grape juice (Tables 48 and 51). However, when 500 mg of A5 was used instead, the recovery of calcium and magnesium was significantly lowered from the unfiltered orange juice (Table 49). The lower recovery of calcium from unfiltered orange juice suggests that the pulp in orange juice may bind Ca and Mg in A5. Carrot juice did not have this problem (Table 54).

TABLE 53

Content of the three elements in fresh carrot juice

Sample	Content (mg/L)		
	Ca	Mg	Zn
Fresh carrot juice	37.499 ± 4.613	75.279 ± 6.183	0.7045 ± 0.0195

Data are expressed as Mean ± S.D. (n=3)

TABLE 54

Elemental recovery from A5 in 330 ml carrot juice stored at 4 °C

	Solubility (g/L)					
	Ca		Mg		Zn	
	2.6 grams	500 mg	2.6 grams	500 mg	2.6 grams	500 mg
Fresh	0.907 ± 0.018	0.196 ± 0.009	0.482 ± 0.013	0.151 ± 0.001	0.0235 ± 0.0022	0.0031 ± 0.0001
One Week	0.935 ± 0.033	0.188 ± 0.006	0.481 ± 0.016	0.150 ± 0.008	0.0491 ± 0.0094**	0.0031 ± 0.0000

Data are expressed as Mean ± S.D. (n=3) ***P<0.01 comparing with fresh group

[0165] This set of studies suggests that A5 can be used to fortify a number of juices and water. The 2.6 g of A5 provides a daily requirement of the three key elements for the prevention of osteoporosis: 300 mg of calcium, 150 mg of magnesium and 5.6 mg of zinc. 500 mg of A5 is intended to provide a serving of these elements in the functional food format.

EXAMPLE 9
Tablet Formulation Compositions

[0166] The relatively low calcium content in A5 has posed a challenge in creating a solid form with a size that is acceptable to end-users. The following formulation was created in tablet form (Table 55):

TABLE 55

Ingredients	Wt./Tablet (mg)	% Comp.	Wt. for 2000 tablets batch (g)
Calcium Acetate blend	550	93.43	1100
Dry Vitamin D_3 100 GFP/HP*	3.25	0.55	6.5
Kollidon VA 64	32.5	5.52	65
Magnesium Stearate	2.93	0.50	5.86
Total	**588.68**	**100.00**	**1177.36**

* Equivalent to 250 IU of Vitamin D_3

[0167] The calcium acetate blend in the above table comprises 14% calcium acetate, 7% magnesium acetate and 0.7% zinc acetate. Magnesium stearate was used as a lubricant.

[0168] The Dry Vitamin D_3 100 GFP/HP composition (as mentioned in the certificate of analysis provided by BASF) is as follows:

Ingredients (CAS No.)	Concentration (w/w)
Sucrose (57-50-1)	30.0 - 40.0%
Starch (9005-25-8)	20.0 - 30.0%
Gum Arabic (9000-01-5)	15.0 - 25.0%
Glycerides (73398-61-5)	5.0 - 15.0%
Water (7732-18-5)	1.0 - 5.0%
Tricalcium phosphate (7758-87-4)	< 1.0%
Vitamin D_3 (67-97-0)	>=0.25%
D,L - alpha - Tocopherol (10191-41-0)	<0.2%

[0169] Assay value: 100,000 IU Vitamin D_3/g (=2500 microgram cholecalciferol/g). The target weight of Vitamin D_3 per tablet is 2.5 mg. 30% extra Vitamin D_3 has been added per tablet as overage. The manufacturer assay value is 100000 IU/g i.e. 100 IU/mg. Since 2.5 mg (3.25 mg with 30% overage) has been used each tablet has ~250 IU of Vitamin D_3.

[0170] The tablets were created according to the following steps:

[0171] **Step 1:** Calcium Acetate blend provided was sieved through 40 mesh screen and 100/120 mesh screen. The fraction that passed through the 40 mesh screen and was retained on 100/120 mesh screen was used for formulation. The fraction of calcium acetate above 40 mesh and below 100 mesh was not used for formulation. This fraction was chosen to keep the particle size similar to other ingredients – Vitamin D_3 and Kollidon Va 64.

[0172] **Step 2: Blending 01:** 6.5 g of dispensed Dry Vitamin D_3 100 GFP/HP and 65 g OF Kollidon VA 64 were blended for 5 minutes at a speed of 25 rpm using a small tumble blender to produce Blend 01.

[0173] **Step 3: Blending 02:** 250 g of dispensed Calcium Acetate blend (Blend 01 * 3.49) prepared in Step 1 was mixed with Blend 01 prepared in Step 2 for 5 minutes to produce Blend 02 (using tumble blender at 25-30 rpm).

[0174] **Step 4: Blending 03:** 250 g of dispensed Calcium Acetate blend prepared in Step 1 was mixed with Blend 02 prepared in Step 3 for 5 minutes to produce Blend 03 (using double cone blender at 25-30 rpm).

[0175] **Step 5: Blending 04:** 600 g of dispensed Calcium Acetate blend prepared in Step 1 was mixed with Blend 03 prepared in Step 4 for 9 minutes to produce Blend 04 (using double cone blender at 25-30 rpm).

[0176] **Step 6: Blending 05:** 5.86 g of dispensed Magnesium Stearate was mixed with Blend 04 prepared in Step 5, for 2 minutes.

[0177] **Step 7:** The final blend prepared above was dispensed using a Rotary table press with target tablet weight of 588.7 g.

[0178] The following formulation was also created in tablet form:

Ingredients	Wt./Tablet (mg)	% Comp.	Wt. for 2000 tablets batch (g)
Calcium Acetate blend	1100	93.43	2200
Dry Vitamin D_3 100 GFP/HP*	6.5	0.55	13
Kollidon VA 64	65	5.52	130
Magnesium Stearate	5.86	0.50	11.72
Total	**1177.36**	**100.00**	**2354.72**

* Equivalent to 500 IU of Vitamin D_3

[0179] The calcium acetate blend in the above table comprises 14% calcium acetate, 7% magnesium acetate and 0.7% zinc acetate. Magnesium stearate was used as a lubricant.

[0180] The Dry Vitamin D_3 100 GFP/HP composition (as mentioned in the certificate of analysis provided by BASF) is as presented above.

[0181] Assay value: 100,000 IU Vitamin D_3/g (=2500 microgram cholecalciferol/g). The target weight of Vitamin D_3 per table is 5 mg. 30% extra Vitamin D3 has been added per tablet to account for loss due to degradation. The manufacturer assay value is 100000 IU/g i.e. 100 IU/mg. Since 5 mg (6.5 mg with 30% overage) has been used each tablet has ~500 IU of Vitamin D_3.

[0182] The tablets were created according to the following steps:

[0183] **Step 1:** Calcium Acetate blend provided was sieved through 40 mesh screen and 100/120 mesh screen. The fraction that passed through the 40 mesh screen and was retained on 100/120 mesh screen was used for formulation. The fraction of calcium acetate above 40 mesh and below 100 mesh was not used for formulation. This fraction was chosen to keep the particle size similar to other ingredients – Vitamin D_3 and Kollidon Va 64.

[0184] **Step 2: Blending 01:** 13 g of dispensed Dry Vitamin D_3 100 GFP/HP and 130 g OF Kollidon VA 64 were blended for 5 minutes at a speed of 25 rpm using a small tumble blender to produce Blend 01.

[0185] **Step 3: Blending 02:** 500 g of dispensed Calcium Acetate blend (Blend 01 * 3.49) prepared in Step 1 was mixed with Blend 01 prepared in Step 2 for 5 minutes to produce Blend 02 (using double cone blender at 25-30 rpm).

[0186] **Step 4: Blending 03:** 500 g of dispensed Calcium Acetate blend prepared in Step 1 was mixed with Blend 02 prepared in Step 3 for 5 minutes to produce Blend 03 (using double cone blender at 25-30 rpm).

[0187] **Step 5: Blending 04:** 1200 g of dispensed Calcium Acetate blend prepared in Step 1 was mixed with Blend 03 prepared in Step 4 for 9 minutes to produce Blend 04 (using double cone blender at 25-30 rpm).

[0188] **Step 6: Blending 05:** 11.72 g of dispensed Magnesium Stearate was mixed with Blend 04 prepared in Step 5, for 2 minutes.

[0189] **Step 7:** The final blend prepared above was dispensed using a Rotary table press with target tablet weight of 1.17 g.

[0190] The size of these two formulations has proven to be acceptable to a test population.

EXAMPLE 10
Gel Cap Formula Consisting of Fish Oil

[0191] A gel cap formula for the Calcium Acetate blend was created to enhance end user acceptance, increased solubility of vitamin D_3 and increased efficacy on bone mineral density.

[0192] Vitamin D_3 is an oil soluble vitamin. It can be dissolved using lipophilic substances.

[0193] Fish oil containing omega 3-6-9 fatty acids is known to have beneficial effects on bone health (32). This oil also has the advantage of dissolving vitamin D_3, obviating the granulation process of Calcium Acetate blend as described in Example 9.

[0194] Fish oil has been found to have the ability to increase the bulk density of Calcium Acetate blend by displacing air from the powder.

[0195] Examples of oil to Calcium Acetate blend ratios include, but are not limited to, about 1:1, 1.5:1 and 2:1.

[0196] Examples of oil to calcium ratios include, but are not limited to, about 1:0.14, 1.5:1 and 2:1.

[0197] Examples of oil to magnesium ratios include, but are not limited to, about 1:0.07, 1.5:0.07 and 2: 0.07.

[0198] Examples of oil to zinc ratios include, but are not limited to, about 1:0.007, 1.5:0.007 and 2:0.007.

[0199] The dosage of vitamin D_3 ranges from 30 to 300 IU.

[0200] Soft gel capsules can be manufactured using conventional methods (33)

[0201] Gel capsules made with this blend in dose sizes amounting to two to four capsules a day will be acceptable. The size of a gel capsules will be equivalent to or smaller than that described in Example 9.

EXAMPLE 11
Optimization of Elemental Formula

[0202] The objective of this example is to design an elemental formula which would provide an optimal mix of vitamin D_3 and acetate salts of calcium, magnesium and zinc for supporting bone health.

[0203] It is a general belief that the bioavailability of calcium is independent of the solubility of calcium salts (Heaney, 1999). Low levels of magnesium and zinc are associated osteoporosis (Mutlu et al., (11). Vitamin D_3 enhances calcium absorption (Christakos et. al., 2011) and therefore, is an important component of an ideal elemental formula.

[0204] Results presented in this invention clearly show that the bioavailability calcium is dependent on the solubility of a calcium salt in the gastrointestinal fluids. An optimal ratio of calcium to magnesium is required to enhance calcium absorption. Vitamin D_3 is responsible for increasing calcium absorption and preventing zinc depletion.

[0205] A formula containing calcium, magnesium, zinc and vitamin D_3 may not work because the form of the elements and the amount of vitamin D_3, are not necessarily formulated in the right ratios in terms of absorbable fractions. The lack of clinical effect of a blend of calcium, magnesium, zinc and vitamin D_3 is a good example (Braam et. al., 2003). The confusion in the literature relating to calcium absorption and the equivocal clinical trial results on bone mineral density by calcium supplementation has created problems for experts skilled in the art in designing an optimal formula of a calcium blend.

[0206] Using the acetate salts of calcium, magnesium and zinc with the appropriate addition of vitamin D_3, an optimum calcium supplement is designed. The ratio of calcium to magnesium is generally 2:1, the ratio of magnesium to zinc is 10:1 and the daily dosage of vitamin D_3 ranges from 500 to 1000 IU.

[0207] The bioavailability of calcium described in this invention is appropriately 2 to 3 times higher than that of Caltrate. The dosage of calcium should be half to one third of that of Caltrate™.

[0208] The recommended intake of calcium from all sources is 1000 mg. The average intake of calcium from dietary sources is 400 mg. It is recommended that 600 mg of calcium should be provided as a supplement; usually this implies that the source of calcium is from calcium carbonate. The recommended dose of calcium from this invention is 200 to 300 mg. This will provide 100 to 150 mg of magnesium and 5 to 7.5 mg of zinc. In addition to dietary intake, the supplementation of magnesium and zinc will also provide an adequate daily requirement of the elements.

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What is claimed is:

1. A method of preparing tablets comprising calcium acetate, magnesium acetate, zinc acetate and vitamin D_3, the method comprises the steps of:
   (i) blending a calcium composition comprising calcium acetate, magnesium acetate, and zinc acetate with a composition comprising vitamin D_3; and
   (ii) blending the composition obtained from (i) with a calcium composition comprising calcium acetate, magnesium acetate, and zinc acetate, thereby obtaining tablets comprising calcium acetate, magnesium acetate, zinc acetate and vitamin D_3.

2. A tablet produced by the method of claim 1.

3. A method of preparing soft gel capsules comprising calcium acetate, magnesium acetate, zinc acetate, fish oil and vitamin D_3, the method comprises the steps of:
   (i) blending a calcium composition comprising calcium acetate, magnesium acetate, and zinc acetate with a composition comprising vitamin D_3 and oil comprising omega 3 or omega 3-6-9 fatty acids; and
   (ii) blending the composition obtained from (i) with a calcium composition comprising calcium acetate, magnesium acetate, and zinc acetate, thereby obtaining soft gel capsules comprising calcium acetate, magnesium acetate, zinc acetate, fish oil and vitamin D_3.

4. The method of claim 1 or 3, wherein the calcium composition comprises at least 10 percent by weight of calcium acetate, at least 5 percent by weight of magnesium acetate, and at least 0.2 percent by weight of zinc acetate.

5. The method of claim 3, wherein the oil is fish oil or flaxseed oil.

6. The method of claim 3, wherein the oil to calcium acetate blend ratio is selected from the group consisting of 1:1, 1.5:1 and 2:1.

7. The method of claim 3, wherein the oil to calcium ratio is selected from the group consisting of 1:0.14, 1.5:1 and 2:1.

8. The method of claim 3, wherein the oil to magnesium ratio is selected from the group consisting of 1:0.07, 1.5:0.07 and 2: 0.07.

9. The method of claim 3, wherein the oil to zinc ratio is selected from the group consisting of 1:0.007, 1.5:0.007 and 2:0.007.

10. A soft gel capsule produced using the method of claim 3.