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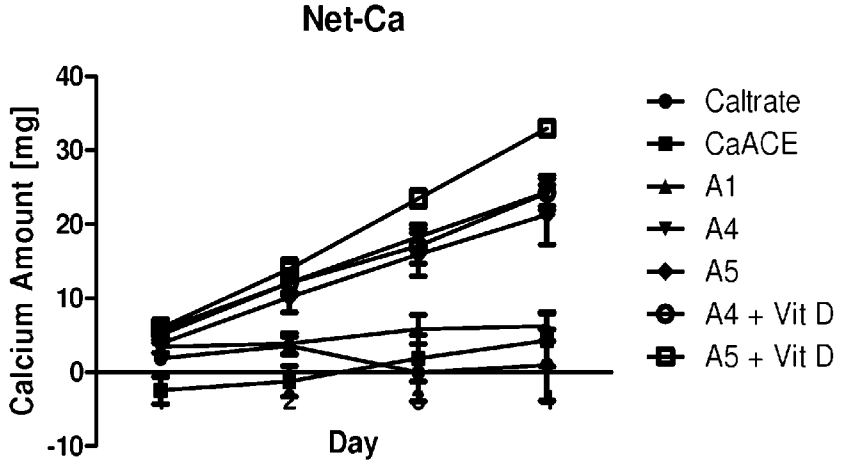
(54) Title: FORMULAS COMPRISING CALCIUM, MAGNESIUM, ZINC, AND VITAMIN D_3 FOR THE PREVENTION AND AMELIORATION OF OSTEOPOROSIS

FIGURE 1



(57) Abstract: The present invention provides formulas of elemental compositions encompassing acetate salts of calcium, magnesium and zinc along with vitamin D_3. The acetate salts could be extracted from natural sources such as pearls, coral, and oyster or compounded using synthetic materials. The dosage and ratio of calcium to magnesium was estimated using in vitro and in vivo estimations. The daily administered amount of calcium for promotion of bone health, prevention, and alleviation of osteoporosis in said formulations is about a quarter to a third of the conventional dose for daily ingestion in conventional osteoporosis treatments and prophylactics.

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FORMULAS COMPRISING CALCIUM, MAGNESIUM, ZINC, AND VITAMIN D_3 FOR THE PREVENTION AND AMELIORATION OF OSTEOPOROSIS

[0001] This application claims benefit of U.S. Provisional Application No. 61/023,997, filed January 28, 2008. The entire contents and disclosures of the preceding application 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.

FIELD OF THE INVENTION

[0003] This invention relates to compositions for the prevention and treatment of osteoporosis. The present invention provides formulas of elemental compositions encompassing acetate salts of calcium, magnesium and zinc along with vitamin D_3. The acetate salts can be extracted from natural sources such as pearls, coral, and oyster or compounded using synthetic materials.

BACKGROUND OF THE INVENTION

[0004] Calcium is the major element in bones. Over 99% of the body's calcium resides in bones. Approximately 80-90% of bone mineral content is comprised of calcium and phosphorus. 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 which leads to increased susceptibility to fracture. This condition often occurs in women age 35 or above. More frequently, it occurs in postmenopausal women (Ilich and Kerstetter, 2000; Ilich et al., 2003).

[0005] Dietary supplementation with calcium was thought to be the prime factor in the maintenance of bone health in the past 50 years (Seelig et al., 2004). However, the benefit of increased calcium consumption has not been clearly demonstrated for bone

health. Instead, high calcium intake may be linked to higher incidence of cardiovascular disease (Seelig et al., 2004). Increased risk of cardiovascular disease was also attributed to a continued increase in calcium to magnesium ratio (Ca/Mg) in the diet. The ratio of Ca/Mg increased from 2/1 from 1900 to 1940 to >3/1 in the 1960s, to >6/1 in 2000. The daily recommended intake (DRI) in 2000 was >3/1 to >4/1.

[0006] There are conflicting reports in the literature concerning the importance of dietary calcium on bone health. Heaney (1993b; Heaney, 1993a) reviewed 43 studies of calcium published between 1988 and 1993. Although 16 studies showed that calcium had no effect on bone loss, 16 of the 19 placebo-controlled studies in which calcium intake was controlled did show that the mineral prevented or slowed bone loss.

[0007] 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 (Riis et al., 1987), all showed that calcium had a significantly beneficial effect.

[0008] In elderly women, it was shown that there was a significant relationship between bone mineral density (BMD) and several critical nutritional parameters: caloric intake provided by macronutrients, protein, calcium, magnesium, zinc and vitamin C (Ilich et al., 2003).

[0009] In the early 2000s, daily calcium intake reached a new high of 2,500 mg (Seelig et al., 2004). It should be noted that the increase in Ca/Mg is mainly due to the increase in calcium intake, not a change in magnesium. The daily requirement of calcium was recently re-evaluated (Hunt and Johnson, 2007). It was found that an average intake of 749 mg of calcium is required, an estimate lower than previously estimated.

[0010] In one clinical trial, 43 early postmenopausal women were randomly assigned to one of the treatment groups: 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. (Riis et al., 1987)

[0011] 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 (Bass et al., 2006). Nutritional intake includes elements such as calcium, phosphorus, magnesium, iron, zinc, sodium and potassium. Among the 7,532 women, 20 years or older, elemental intake was not a predictor of osteoporosis. This observation may not be surprising because the average calcium intake was 659 mg and magnesium was 241 mg. These values were lower than that of RDA of 1000 and 310 mg, respectively.

[0012] Physical activity was associated with increase in vertebral bone mineral density (Kanders et al., 1988). When activity was removed, vertebral bone mineral density was dependent on calcium intake. However, when calcium intake exceeded 800 to 1000 mg/day vertebral bone mineral density was not dependent on calcium intake in the absence of exercise. Bone mineral density reached a plateau when calcium intake was 800 to 1000 mg/day with no additional benefit accruing to the additional consumption of calcium. Such a ceiling effect of calcium was also observed by Celotti and Bignamini (1999). They reported that calcium supplementation is important for maintaining bone health. However, excessive amount of calcium may be useless and it could cause hypercalciuria and kidney stones. Supplementation with a small amount of magnesium was suggested.

[0013] Mutlu et al. (2007) showed that magnesium and zinc levels are the lowest in postmenopausal women with osteoporosis as compared to postmenopausal women with osteopenia, or postmenopausal women with normal bone density. Calcium

supplementation may reduce zinc absorption, and magnesium and zinc retention. These conditions further aggravate the severity of osteoporosis (Ilich and Kerstetter, 2000; Lowe et al., 2002; Abrams and Atkinson, 2003). Besides calcium, magnesium, zinc, manganese and copper deficiencies are linked to osteoporosis (Saltman and Strause, 1993).

[0014] To determine the influence of diet on bone mass in the proximal femur, Angus et al. (1988) measured 14 nutrients in 159 Caucasian women aged 23-75 and bone mineral density (BMD) in the hip, spine and forearm. No significant correlation was found between calcium intake and bone mass at any site. Iron, zinc and magnesium were positively correlated with forearm BMD in premenopausal women. Iron and magnesium were significant predictors of forearm BMD in premenopausal and postmenopausal women respectively by multiple regression analysis.

[0015] A study emphasizing the benefit of magnesium on postmenopausal women found that a Mg/Ca ratio of 1.2/1 was more effective than that of a ratio of 0.4/1 (Abraham and Grewal, 1990). 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 11% increase in bone mineral density, whereas, the other group had a non-significant increase of 0.7%.

[0016] Magnesium supplementation (250 mg/day) in young women has been shown to have no effects on calcium resorption (Basso et al., 2000). This study was a short term study. Therefore, the validity of the results is yet to be confirmed.

[0017] Ilich (2000) wrote, “Osteoporosis is a complex, multi-factorial condition characterized by reduced bone mass and impaired micro-architectural structure, leading to an increased susceptibility to fractures. Although most of the bone strength (including bone mass and quality) is genetically determined, many

other factors (nutritional, environmental and life-style) also influence bone. Nutrition is an important modifiable factor in the development and maintenance of bone mass and the prevention and treatment of osteoporosis. Approximately 80-90% of bone mineral content is comprised of calcium and phosphorus. Other dietary components, such as protein, magnesium, zinc, copper, iron, fluoride, vitamins D, A, C, and K are required for normal bone metabolism, while other ingested compounds not usually categorized as nutrients (e.g. caffeine, alcohol, phytoestrogens) may also impact on bone health. Unraveling the interaction between different factors; nutritional, environmental, life style, and heredity help us to understand the complexity of the development of osteoporosis and subsequent fractures. This paper reviews the role of dietary components on bone health throughout different stages of life. Each nutrient is discussed separately; however the fact that many nutrients are co-dependent and simultaneously interact with genetic and environmental factors should not be neglected. The complexity of the interactions is probably the reason why there are controversial or inconsistent findings regarding the contribution of a single or a group of nutrients in bone health."

[0018] 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.

[0019] Despite the values cited in the Recommended Daily Allowance (RDA), Allowable Intake (AI) or Recommended Daily Intake (RDI) for elemental intake, there was not much attention paid to the form of elements consumed. It is not clear whether calcium salts can be used interchangeably. It is understandable that not all calcium salts are created alike; there are differences in solubility and absorption. If there are differences in bioavailability, absorbability of elements from salts should be measured more accurately.

[0020] These issues have not received appropriate attention because there are reports showing 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 (Bo-Linn et al., 1984). The hypothesis that calcium carbonate can be converted to a more soluble calcium salt, calcium chloride in the stomach, which enhances calcium absorption has been tested. The results showed that calcium carbonate absorption is not influenced by gastric acid (Bo-Linn et al., 1984).

[0021] 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 (Tsugawa et al., 1995). 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. Accurate comparison of calcium bioavailability cannot be achieved using an indirect method such as PTH.

[0022] Heaney (2001) reported that rates of urinary excretion for three marketed calcium products (marketed calcium carbonate, encapsulated calcium carbonate and marketed calcium citrate) were identical. 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 (Heaney et al., 1999).

[0023] Magnesium absorption from 10 organic and inorganic salts was tested in rats (Coudray et al., 2005). The bioavailability of magnesium ranged from 50 to 66%. Magnesium gluconate provided the highest value. Solubility of these salts in the small and large intestine and cecum was measured. Solubility of these salts is actually quite high at the proximal section of the intestine; it dropped off very quickly as pH increased along the intestinal tract. Differences in these magnesium salts'

absorption may not be important considering the variability among individuals.

[0024] Bioavailability of elements in fortified foods has been measured using dual isotope techniques (Abrams et al., 2002). There was no difference in the bioavailability of zinc oxide and zinc sulfate; both are 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. The absorption of calcium in fortified cereal was 28.9%; in unfortified cereal was 30.8 %.

[0025] 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 (Tsugawa et al., 1999). The bioavailability was measured using ^{45}Ca. Solubility of these salts under different pH conditions was also measured.

[0026] Bioavailability of calcium acetate was measured using ^{45}Ca (Cai et al., 2004). Compared to calcium ascorbate, bioavailability of calcium acetate was significantly lower (70% vs. 45% at 25 mg calcium load). A kinetic model comprising 8 compartments was used to fit the plasma calcium vs. time data. The difference between bioavailability of calcium ascorbate and bioavailability of calcium acetate was attributed to a difference via 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.

[0027] Ten mg of zinc per day is the recommended intake (Record et al., 1985). The recommended daily allowance of zinc was 6 mg (Smith et al., 1983). The authors warned that recommended daily allowance should not be confused with that of recommended daily intake.

[0028] Zinc absorption occurs throughout the small intestine and it is dose dependent in humans (Lee et al., 1989).

[0029] A patent was filed in 1999 for a calcium dietary supplement comprising calcium, magnesium, zinc, etc. (Ellenbogen and Buono, 1999). The range of calcium used was 1000 to 2500 mg 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. Furthermore, the calcium to magnesium ratios are high (6.7 to 25:1) compared to the range described in this invention.

[0030] Meigant and Stenger (2004) filed a U.S. patent citing the a composition which consists of calcium and a vitamin D mixture The patent describes preparation of galenic formulations of calcium and vitamin D using combinations of excipients; therefore, the thrust of the present application shares no common ground with the application of Meigant and Stenger (2004).

[0031] Hendricks (2004) was awarded a patent on 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 perhaps vitamin D, vitamin B_6 and vitamin B_{12}. The present application, however, does not include phosphorus.

[0032] Mazer et al. (1997) was granted a patent on a calcium supplement in solid form which contains calcium glycerophosphate, vitamin D and vitamin C. Again, the present invention does not contain calcium salt of this kind.

[0033] In another patent, the synthesis of dicalcium citrate-lactate was described by mixing stoichiometric mixtures of citrate and lactate salts to produce the calcium salt (Walsdorf et al., 1991).

[0034] Krumhar and Johnson (2006) designed a diet supplement for bone health consisting of microcrystalline calcium hydroxyapatite, protein (mostly collagen), phosphorus, fat, and other minerals. It also contains vitamin D_3 from 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. A method for benefiting human bone health involves administering a daily regimen of the dietary supplement.

[0035] There is 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 (Sultenfuss, 1996). Ca/Mg ratio is 1000-1500/400-600.

[0036] A dietary supplement for supplementing dietary needs of women and preventing or reducing life stage associated health risks during each of their principal adult life stages (pre-perimenopausal, perimenopasue and menopause, or post-menopause) consisting of an extensive list of minerals and vitamins was described in a patent (Jackson and Blumberg, 1997). This list of nutrients was derived from various sources and there were no data to support individual claims. Although there were specific amounts of elements cited for osteoporosis support, the forms of salt were not specified. If different forms of elements such as carbonate, oxide, acetate, etc. were used, the performance of these nutrients could be drastically different because their bioavailability varies.

[0037] Much attention has been focused on calcium as the element for bone health. However, not all calciums are the same and their relative bioavailability determines the fractional amount that reaches the systemic circulation. As for maintenance of bone health, other essential elements are required. There are hints in the literature suggesting that potential interactions between these elements exist. The impact on absorption, calcium utilization and consequently, bone health has not been systematically investigated. Furthermore, vitamins such as D_3 and K_2 have been implicated in calcium absorption and increase in bone mineral density (BMD); however, the interplay between bioavailable elements, such as calcium, magnesium and zinc, with vitamins has not been illustrated. The complicated environment in the gastrointestinal tract plays a dominant role in determining the absorbability of elements. In particular, cations and anions may play a significant role in altering the solubility of an elemental salt in the gastrointestinal tract (GIT). The importance of these factors in determining the bioavailability of elements has never been thoroughly addressed. In this invention, a calcium supplement, comprising optimum amounts of acetate salts of calcium, magnesium and zinc, and vitamin D_3, is described. The daily dosage of calcium is significantly lower than that of regular calcium supplement. This product was designed using in vitro and in vivo models which are key to determining elemental balance.

BRIEF SUMMARY OF THE INVENTION

[0038] The present invention provides a dietary supplement comprising acetate salts of calcium, magnesium, zinc and vitamin D_3. This preparation is highly soluble in water, gastric and intestinal fluids. It is also shown that elemental absorption is high and the calcium dosage required for bone health maintenance is approximately a quarter to a third of that of the conventional calcium dose.

DETAILED DESCRIPTION OF THE FIGURES

[0039] **Figure 1** shows the average cumulative net amount of calcium retained (±S.E.M.) in rats receiving a calcium free diet over a four day period. The seven groups of animals which participated in this study were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a suspension, while animals in the other groups received a solution. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5. The dosage of vitamin D_3 was 0.8 IU/mg calcium.

[0040] **Figure 2** shows the average cumulative net amount of magnesium retained (±S.E.M.) in rats receiving calcium free diet over a four day period. The seven groups of animals which participated in this study were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a suspension, while animals in the other groups received a solution. Calcium acetate (CaACE) contained 23.3% Calcium. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5. The dosage of vitamin D_3 was 0.8 IU/mg calcium.

[0041] **Figure 3** shows the average cumulative net amount of zinc retained (±S.E.M.) in rats receiving calcium free diet over a four day period. The seven groups of animals which participated in this study were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a suspension, while animals in the other groups received a solution. Calcium acetate (CaACE) contained 23.3% Calcium. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64%

Magnesium and 0.57% Zinc for A5. The dosage of vitamin D_3 was 0.8 IU/mg calcium.

[0042] **Figure 4** shows the plasma calcium (A), magnesium (B) and zinc (C) levels sampled from rats at the end of the treatment period while receiving a calcium free diet. Seven groups of animals participated in this study; the groups were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a suspension, while animals in the other groups received a solution. Calcium acetate (CaACE) contained 23.3% Calcium. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5. The dosage of vitamin D_3 was 0.8 IU/mg calcium.

[0043] **Figure 5** shows the average cumulative net amount of calcium retained (±S.E.M.) in rats receiving normal diet over a four day period. The five groups of animals which participated in this study were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a suspension, while animals in the other groups received a solution. Calcium acetate (CaACE) contained 23.3% Calcium. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0044] **Figure 6** shows the average cumulative net amount of magnesium retained (±S.E.M.) in rats receiving normal calcium diet, as described on Table 34, over a four day period. The five groups of animals which participated in this study were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a

suspension, while animals in the other groups received a solution. Calcium acetate (CaACE) contained 23.3% Calcium. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0045] **Figure 7** shows the average cumulative net amount of zinc retained (±S.E.M.) in rats receiving normal calcium diet, as described on Table 34, over a four day period. The five groups of animals which participated in this study were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a suspension, while animals in the other groups received a solution. Calcium acetate (CaACE) contained 23.3% Calcium. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0046] **Figure 8** shows the average plasma calcium (A), magnesium (B) and zinc (C) levels sampled from rats on Day 5, the end of the treatment period, while receiving normal calcium diet. Blood samples were taken 1.5 hours after the last treatment. The five groups of animals which participated in this study were named after the treatment formulations. The dosage of calcium for animals in each group was 53 mg/kg. Each animal received 1 mL of treatment daily. Animals in the Caltrate™ group received a suspension, while animals in the other groups received a solution. Calcium acetate (CaACE) contained 23.3% Calcium. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0047] **Figure 9** shows the average 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. There were three groups of animals participated in this study. Each animal received treatment once a day. The average dosage of

calcium for each treatment group was 625 mg/kg/day. Animals in the DI group received a normal calcium diet (Table 34), while A1 and A5 group received a calcium free diet (Table 34). One mL of distilled water (DI) was administered to each member of the DI group by gavage and one mL of treatment solution was administered to the other group members by gavage. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0048] **Figure 10** shows average 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. There were three groups of animals participated in this study. Each animal received treatment once a day. The average dosage of calcium for each treatment group was 625 mg/kg/day. Animals in the DI group received a normal calcium diet (Table 34), while A1 and A5 group received a calcium free diet (Table 34). One mL of distilled water (DI) was administered to each member of the DI group by gavage and one mL of treatment solution was administered to the other group members by gavage. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0049] **Figure 11** shows the average cumulative net amount of zinc retained (±S.E.M.) in rats receiving a calcium free diet plus a daily consumed dose of calcium over a four day period. There were three groups of animals participated in this study. Each animal received treatment once a day. The average dosage of calcium for each treatment group was 625 mg/kg/day. Animals in the DI group received a normal calcium diet (Table 34), while A1 and A5 group received a calcium free diet (Table 34). One mL of distilled water (DI) was administered to each member of the DI group by gavage and one mL of treatment solution was administered to the other group members by gavage. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0050] **Figure 12** 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. There were three groups of animals participated in this study. Each animal received treatment once a day. The average dosage of calcium for each treatment group was 625 mg/kg/day. Animals in the DI group received a normal calcium diet (Table 34), while A1 and A5 group received a calcium free diet (Table 34). One mL of distilled water (DI) was administered to each member of the DI group by gavage and one mL of treatment solution was administered to the other group members by gavage. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5.

[0051] **Figure 13** is the body mass record of 15 groups of rats which received individual elemental treatments. The treatment groups were named after their respective treatments. All animals received a normal calcium diet (Table 34) *ad libitum* and a once a day dose of treatment containing 53 mg/kg of calcium. Formulations containing Caltrate™ were administered as suspensions; whereas the others solutions. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5. The dosages of vitamin D_3 and vitamin K_2 were 0.8 IU/mg calcium and 12.75 IU/kg/day, respectively. Bisphosphonate was given as alendronate and the dosage is: 14 μg/kg/2-week.

[0052] **Figure 14** shows trabecular BMD of Distal Femur Averaged from 3 pQCT Slices. *: significantly different from OVX-control ($p<0.05$). Fifteen groups of rats which received individual elemental treatments. The treatment groups were named after their respective treatments. All animals received a normal calcium diet (Table 34) *ad libitum* and a once a day dose of treatment containing 53 mg/kg of calcium. Formulations containing Caltrate™ were administered as suspensions; whereas the others solutions. The elemental composition was 23.3% Calcium,

0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5. The dosages of vitamin D_3 and vitamin K_2 were 0.8 IU/mg calcium and 12.75 IU/kg/day, respectively. Bisphosphonate was given as alendronate and the dosage is: 14 µg/kg/2-week.

[0053] Figure 15 shows trabecular BMD of Proximal Tibia Averaged from 3 pQCT Slices. *: significantly different from OVX-control ($p<0.05$). Fifteen groups of rats which received individual elemental treatments. The treatment groups were named after their respective treatments. All animals received a normal calcium diet (Table 34) *ad libitum* and a once a day dose of treatment containing 53 mg/kg of calcium. Formulations containing Caltrate™ were administered as suspensions; whereas the others solutions. The elemental composition was 23.3% Calcium, 0.0012% Magnesium and 0.33% zinc for A1, 7.51% Calcium, 7.5% Magnesium and 0.38% Zinc for A4 and 11.5% Calcium, 5.64% Magnesium and 0.57% Zinc for A5. The dosages of vitamin D_3 and vitamin K_2 were 0.8 IU/mg calcium and 12.75 IU/kg/day, respectively. Bisphosphonate was given as alendronate and the dosage is: 14 µg/kg/2-week.

DETAILED DESCRIPTION OF THE INVENTION

[0054] Definitions:

[0055] "Bioavailable" is defined as the ability of a drug or a substance to be absorbed and used by the body

[0056] "Bioavailability" is defined as the rate and extent of a substance reaching systemic circulation.

[0057] The present invention describes a supplement comprising acetate salts of calcium, magnesium, zinc and vitamin D_3.

[0058] In one embodiment of the composition, there exists a weight ratio of calcium to magnesium of 0.5:1 to 4:1 For example, the composition of the present invention may comprise 220 mg of calcium and between 55 mg to 440 mg of magnesium. In a preferred embodiment of the composition, there exists a weight ratio of calcium to magnesium of 1:1 to 2:1. In another embodiment, the composition of the present invention comprises a weight ratio of zinc to calcium ranging from about 0.05:1 to about 0.2:1. In a preferred embodiment, the composition of the present invention comprises a weight ratio of zinc to calcium ranging from about 0.05:1 to about 0.1:1.

[0059] In another embodiment of the composition, there exists 40 to 1200 IU of vitamin D_3 per 220 mg of calcium.

[0060] In another embodiment, the daily dose of the elements to be administered to an adult human patient comprises the following: 50 to 500 mg of calcium, 400 to 1200 IU of vitamin D_3, and 5 to 40 mg of zinc. In another embodiment, such a daily dose may be administered only once daily or fractional portions of the daily dose can be administered in separate allotments throughout the day. In another embodiment, an amount of the composition making up the daily dose can be dissolved in water or fruit juice for consumption. In another embodiment, an amount of the composition

comprising the entire daily dose may be encapsulated and administered to an adult human patient. In another embodiment, a fractional portion of the daily dose can be encapsulated and administered in separate allotments throughout the day until the entire daily dose is administered.

[0061] The present invention also provides a method of alleviating symptoms of osteoporosis in humans or animals, comprising the step of administering the composition of the present invention to humans or animals. In one embodiment, the composition comprises between 50 to 500 mg of calcium per daily dose administered to a human adult. In a preferred embodiment, the composition comprises 110 mg to 220 mg of calcium per daily dose administered to a human adult.

[0062] The present invention also provides a method of increasing bone mineral density in humans or animals, comprising the step of administering the composition of the present invention to humans or animals. In one embodiment, the composition comprises between 50 to 500 mg of calcium per daily dose administered to a human adult. In a preferred embodiment, the composition comprises 110 mg to 220 of calcium per daily dose administered to a human adult.

[0063] The present invention provides a composition comprising an initial composition of at least 22.75 percent calcium by weight extracted from calcium containing or synthetic calcium acetate-containing sources, wherein said initial composition is fortified with magnesium and zinc to provide a final composition of at least 4 percent by weight of calcium, at least 5 percent by weight of magnesium, at least 0.2 percent by weight of zinc, and at least 400 IU of vitamin D_3.

[0064] The present invention provides a composition comprising an initial composition of at least 22.75 percent calcium by weight extracted from calcium containing or synthetic calcium acetate-containing sources, wherein said initial composition is fortified with magnesium and zinc to provide a final composition

of at least 4 percent by weight of calcium, at least 5 percent by weight of magnesium, at least 0.2 percent by weight of zinc, and at least 400 IU of vitamin D_3 and wherein the calcium containing sources are pearls, corals, oysters, or natural ores.

[0065] The present invention provides for compositions where the magnesium is in the form of acetate salt.

[0066] The present invention provides for compositions where the zinc is in the form of acetate salt.

[0067] The present invention provides for compositions comprising a weight ratio of calcium to magnesium of 0.5:1 to 4:1. The present invention further provides for compositions comprising a weight ratio of calcium to magnesium of 1:1 to 2:1.

[0068] The present invention provides for compositions comprising a weight ratio of zinc to calcium ranging from 0.05:1 to 0.20:1. In a preferred embodiment, the present invention further provides for compositions comprising a weight ratio of calcium to magnesium of 0.05:1 to 0.1:1.

[0069] The present invention provides for compositions comprising 5-40 mg of zinc per daily dose to be administered to a human adult.

[0070] The present invention provides for compositions comprising 400 to 1200 IU of vitamin D_3 per daily dose to be administered to a human adult.

[0071] The present invention provides for compositions wherein the composition comprises 50 to 500 mg of calcium and 25 to 500 mg of magnesium per daily dose to be administered to a human adult. In a preferred embodiment, the present invention also provides for compositions wherein the composition comprises 100 to 300 mg of calcium and 50 to 150 mg of magnesium per daily dose to be administered to a human adult.

[0072] The present invention provides for compositions wherein the composition comprises 400 to 1200 IU of vitamin D_3 per 220 mg of calcium per daily dose to be administered to a human adult.

[0073] The present invention provides for compositions wherein said composition comprises more bioavailable calcium per unit weight than calcium carbonate.

[0074] The present invention provides for a method of alleviating or preventing symptoms of osteoporosis in humans or animals, comprising the step of administering the recited compositions to said humans or animals.

[0075] The present invention provides for a method of preventing or alleviating symptoms of osteoporosis in humans or animals wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult. In a preferred embodiment, the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

[0076] The present invention provides for a method of increasing bone mineral density in humans or animals, comprising the step of administering the recited compositions to said humans or animals.

[0077] The present invention provides for a method of increasing bone mineral density in humans or animals, wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult. In a preferred embodiment, the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

[0078] The present invention provides for the use of any of the compositions recited to prevent or alleviate symptoms of osteoporosis.

[0079] The present invention provides for the use of any of the compositions recited to alleviate symptoms of osteoporosis wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult. In a preferred embodiment, the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

[0080] The present invention provides for the use of any of the compositions recited to increase bone density.

[0081] The present invention provides for the use of any of the compositions recited to increase bone density, wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult. In a preferred embodiment, the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

[0082] This invention provides for the use of any of the compositions recited to facilitate dietary calcium and magnesium absorption.

[0083] This invention provides for the use of any of the compounds recited to facilitate dietary calcium and magnesium absorption, wherein the composition comprises between 50 to 500 mg of calcium. In a preferred embodiment, the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

[0084] The invention being generally described, will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

EXAMPLE 1

Formulations of Pearl Extracts

[0085] A pearl extract was prepared by adapting the patented method reported by Li and Li. 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 in 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

[0086] 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

[0087] 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.

EXAMPLE 2

Solubility of Calcium In Artificial Gastric and Intestinal Juice

[0088] The objective of this study was to measure and compare the solubility of acetate formulas and Caltrate™ in artificial gastric and artificial intestinal juice (United States Pharmacopoeia).

[0089] Artificial gastric fluid was prepared by dissolving 2.0 g of sodium chloride in 1.0 L of de-ionized water containing 7.0 mL of concentrated hydrochloric acid. Artificial intestinal fluid was prepared by dissolving 6.8 g of monobasic potassium phosphate (KH_2HPO_4) in 1.0 L of de-ionized water containing 77 mL of 0.2 N sodium hydroxide.

[0090] Excess amount of each formula was added to a predetermined volume of artificial gastric or intestinal juice. The mixture was stirred at 37 °C for an hour. An aliquot of the saturated solution was sampled and diluted before measurement.

[0091] 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.

[0092] 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 GIT fluids.

[0093] 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. The solubility of magnesium salt in Caltrate™ is low. The solubility of magnesium in Caltrate™ is also higher in artificial gastric juice.

[0094] The solubility profile of zinc salts is also similar to that of magnesium and calcium, except the magnitude of difference between salt forms and environmental conditions is less drastic (Table 5).

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

[0095] 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 3 - 5 highlight some of the physiological factors which have been postulated to have a significant impact on the solubility of elements. In terms of solubility, the response of four test formulas (A1, A4, A5 and A6), Caltrate™ and calcium acetate to pH, anions and cations that are present in abundance in GIT fluids was evaluated.

[0096] In this example, the effects of pH (ranging from 1 to 9) on the solubility of three elements of 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 mixture was 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 P value was set at 0.05.

[0097] 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).

[0098] 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).

[0099] 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 than that at pH 2 and 3.

[00100] pH may become an issue for calcium absorption when Caltrate™ is administered because intestinal pH values are higher than 6. Under this condition, the solubility of calcium carbonate in Caltrate™ is really low. These results are consistent with that reported on 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

EXAMPLE 4

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

[00101] 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

[00102] Tables 9-11 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 9-11). 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 9
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 10
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 11

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

[00103] 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 9 and 12). 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.

[00104] In the presence of chloride, pH has less of an effect on magnesium solubility (compare values between Tables 10 and 13). In general, the solubility of magnesium at pH 7 is slightly lower for all formulas and the chloride effect is not pronounced.

[00105] 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 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 12

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 13

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 14

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.

[00106] The solubility of calcium in Caltrate™ increased with the increase of bicarbonate concentration (Table 15). 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%.

[00107] The solubility of magnesium in Caltrate™ increased with bicarbonate concentration (Table 16). Bicarbonate effect was minimal for the acetate formulas.

[00108] The solubility of zinc in Caltrate™ increased in the presence of bicarbonate (Table 17). 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 15
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 16
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 17
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

[00109] Phosphates have insignificant effects on the solubility of calcium in Caltrate™ (Table 18). As phosphate concentrations increased the solubility of calcium decreased in all acetate formulations. Maximum reduction (up to 40%) 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.

[00110] Magnesium solubility decreased as phosphate concentration increased (Table 19). 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.

[00111] Among the three elements, phosphates have the most intense effect on the solubility of zinc (Table 20). 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 18
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 19
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 20
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

A. Effects of Na^+ at pH 1

[00112] 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 21 and 22 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 23).

TABLE 21

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	84.800 ± 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 22

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 23
A 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

[00113] Tables 24-26 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 24
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 25
A 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 26
A 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

[00114] There is a tendency for the solubility of calcium to increase with an increase in potassium ion concentration (Table 27). 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 ($p<0.05$).

[00115] Magnesium solubility profiles show a similar trend (Table 28) to that of calcium. The most pronounced was that measured for Caltrate™, a three-fold increase ($p<0.05$). This trend was not significant for all the acetate formulas.

[00116] Zinc solubility tended to increase with an increase in potassium concentration (Table 29). The most pronounced increase was obtained from the zinc in Caltrate™. A similar trend was observed for calcium acetate. The trend was insignificant for the pearl extract formulas ($p>0.05$).

TABLE 27

A 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 28
A 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 29
A 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

[00117] 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 30). No calcium could be detected in preparations using Caltrate™.

[00118] 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 31-32). No measurable magnesium and zinc was reported for preparations using Caltrate™.

TABLE 30

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 31

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 32
A 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.

TABLE 33
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 6

In Vivo Evaluation of Calcium, Magnesium And Zinc Balance

[00119] 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

[00120] Two diets, one with normal calcium and the other is calcium free, were used for the studies. The nutrient composition of the diets are 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) 6.0 (acid hydrolysis)	10.0
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

[00121] 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 began. 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

[00122] There were two set of studies 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: Caltrate™, Calcium Acetate (Ca ACE), A1, A4, A5, A4 plus vitamin D_3 and A5 plus vitamin D_3 (n = 5 per group). Rats participated in the normal diet study received normal diet *ad libitum* throughout. Rats participated 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. Contents 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. Average daily dietary intake of calcium, magnesium and zinc was 625, 155 and 10 mg/kg/day, respectively. Daily elemental dosages 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; 1IU=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

[00123] 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. Each animal was anesthetized shortly before peak blood collection with a heparinized syringe via cardiac puncture. Immediately after blood collection, the animal was then sacrificed with an over dose 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 collected from the metabolism cage and volume 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.

[00124] Daily calcium balance was calculated using equation 1:

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

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

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

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.

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

[00125] 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

[00126] 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.

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.

[00127] 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). From the calcium retention standpoint, it appears a 2/1 Ca/Mg ratio is optimal.

[00128] The addition of vitamin D_3 increases calcium retention significantly (Figure 1 and Table 36). Calcium retention increased to 62% when vitamin D_3 was added to A5 (Table 36).

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.

[00129] Magnesium appears to be required in order to maintain magnesium balance (Table 37). Formulas (Caltrate™, CaACE and A1) that have miniscule amounts of magnesium caused a net loss of magnesium (Figure 2 and Table 37).

[00130] 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 2 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.

[00131] The retention of zinc is highly variable; it is particularly true with formulas such as Caltrate™, calcium acetate and A1 that contain minute amount of zinc (Table 38). The results also show that zinc balance became negative when the amount of zinc is low.

[00132] 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 3).

[00133] However, the addition of vitamin D_3 to A4 and A5 made zinc balance positive (Figure 3 and Table 38). The importance of vitamin D_3 on zinc is clearly demonstrated in this set of study.

[00134] Figure 4 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

[00135] 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.

[00136] 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 5 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	31.873 ± 3.0
A1	31.4 ± 33.5$	49.2 ± 38.8$	39.2 ± 27.3	31.306 ± 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

[00137] Average dietary intake of magnesium by the study animals was approximately 35 mg. Magnesium balance for all study groups was positive (Figure 6 and Table 41). This observation is consistent with the observation obtained from animals receiving calcium free diet, which contained very low quantity of magnesium (Tables 37 and 41). The calcium free diet study showed that magnesium intake was required for positive magnesium balance (Table 37). The presence of magnesium in the elemental formulas did not significantly alter magnesium balance (Table 41). However, the day to day trend showed that animals treated with acetate formulas (CaACE, A1, A4 and A5 vs. Caltrate™) has consistently higher net percentage of magnesium.

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

[00138] There were no statistical differences among elemental treatments in terms of zinc balance (Figure 7 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 7 and Table 42). This observation is similar to that observed in the calcium free diet study (Table 38).

[00139] 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.

[00140] Figure 8 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

[00141] 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 ($p<0.001$).

[00142] Consistent with the results obtained from the normal and calcium free diet studies, magnesium has a minor effect in enhancing calcium retention (Figure 9 and Table 44). The administration of a soluble form of calcium, calcium acetate, significantly enhanced calcium balance (Figure 9 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

[00143] Consistent with the calcium free diet study described above, magnesium was required to maintain a positive magnesium balance (Figure 10 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

[00144] 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 11 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 11 and Table 46); suggesting high solution concentration of calcium in the intestine may interfere with zinc absorption.

[00145] Figure 12 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

[00146] 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.

[00147] 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.

[00148] 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.

[00149] 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.

[00150] 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 ($p<0.05$).

[00151] Body weight changes for different treatment groups are shown in Figure 13 and Table 47. As expected, weight gains in the OVX rats were significantly greater than the normal rats ($p<0.05$).

Table 47

Body Weight On The First Day And The Last Day Of Treatment

	Day 1	Day 57
CONTROL-SHAM	317±14	344±13
CONTROL-NO OVX	278±24	294±23
A1	339±32	371±34
Caltrate	317±22	338±32
A1+VD	340±43	372±49
A4+VD	337±31	371±34
A5+VD	322±26	342±35
A6+VD	337±29	371±39
A4+VD+K	328±19	363±9
A5+VD+K	298±31	331±38
Catrate+VD+ K	270±25	301±36
BIS+A1+VD	336±20	358±27
BIS+A4+VD	338±14	371±28
BIS+A5+VD	321±34	351±43
BIS+Cal+VD	340±48	368±58

[00152] 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 14), 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 15). 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).

[00153] 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.

[00154] 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

Optimization of Elemental Formula

[00155] 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.

[00156] In the study reported by Seelig et al., a high Ca/Mg ratio in a diet is associated with osteoporosis and unwanted cardiovascular events. The high dietary calcium intake in the last 50 years may be undesirable. Other investigators have shown that dietary calcium intake may not be a significant factor in determining bone density and therefore, osteoporosis in older men and women. Magnesium has been identified to be an important element in bone metabolism because it is essential for a number of enzymes which are involved in bone metabolism. Furthermore, when patients are diagnosed with osteoporosis, they invariably have low serum levels of magnesium. In addition, when dietary calcium is shown to improve bone density, magnesium is always present in a significant quantity.

[00157] The question is: What is the optimal ratio of calcium to magnesium? Are the ratios important? Seelig et al. found that a Ca/Mg ratio of 2/1, a dietary composition in the early 1900s, was associated with the least cardiovascular diseases. However, the optimal ratio of calcium to magnesium has not been carefully evaluated. An issue that needs to be addressed is the variability in solubility of calcium and magnesium salts. As shown in Table 3, difference in calcium solubility in artificial intestinal fluid could amount to over 25,000 fold. The relationship between solubility and bioavailability of calcium is generally considered to be unimportant, as it was demonstrated by several research groups. However, the number of calcium salts used was limited. In a study reported by Hanzlik et al., the solubility of calcium in the intestine plays a significant role in the bioavailability of calcium. Our animal results support the notion that calcium absorption is highly dependent on the salt form. The difference in bioavailability between calcium carbonate and calcium acetate could amount to 3-fold.

[00158] Interestingly, the story with magnesium is very similar. The reported range of magnesium bioavailability ranged from 50 to 67%.

[00159] The only study that evaluated the effect of calcium and Ca/Mg ratio on bone density in postmenopausal women was performed by Abraham and Grewal (1990). The finding was that a ratio of Ca/Mg of 1/1.2 was significantly better than that of 1/0.4. The amount of calcium used in the study was 500 mg. The calcium salt used was calcium citrate and the magnesium salt used was magnesium oxide. According to the literature, the bioavailability of calcium citrate is 30% and it was not different from that of calcium carbonate (Heaney et al., 1999). The bioavailability of magnesium oxide is 50% (Coudray et al., 2005). If Ca/Mg ratio was to be calculated using bioavailable doses of calcium and magnesium, the Ca/Mg ratio employed by Abraham and Grewal (1990) would have been 1/2. The Abraham and Grewal study (1990) has established that magnesium is important

in preventing osteoporosis. However, there was no definitive ratio set for Ca/Mg. This could be due to: a. the dosage of calcium; b. the availability of individual calcium salts; and c. the actual absorbable quantity of calcium and magnesium.

[00160] Our results show that there is a complex interplay between calcium, magnesium, zinc, vitamin D_3 and nutritional status on elemental balance (Examples 3 to 6).

[00161] Factors such as pH, cation and anion concentrations have different impacts on the solubility of calcium salts (Examples 3 to 5). The solubility of calcium in the form of calcium carbonate is extremely low under various experimental conditions, suggesting that the absorption of calcium will be low because the salt is not soluble along the entire GIT. The solubility of calcium in the form of calcium acetate is high and it is not affected significantly by pH, cations and anions. Cations tend to increase its solubility, but anions, such as bicarbonates, chloride and phosphate tend to reduce calcium solubility (Examples 3 to 5). Since the concentrations of cations and anions tested were within the physiological range (Table 33) and since there are opposing effects contributed by cations and anions, it is anticipated that that calcium acetate will remain in solution along GIT.

[00162] Magnesium is shown to enhance calcium absorption and balance (Example 6). Conversely, calcium and magnesium tend to diminish zinc balance. The intensity of the interplay is dependent on nutritional status of the animal. These complex interplays between the three elements can be nullified by the addition of vitamin D_3.

[00163] It has been suggested that dosage of calcium used for the past decades is too high and it should be trimmed to 750 mg. Since calcium carbonate is the most common form of calcium administered, it is equivalent to 180 mg of absorbable calcium, assuming a 24% bioavailability (Bo-Linn et al., 1984). The daily magnesium requirement is 310 mg and it is equivalent to 155 to

186 mg of bioavailable magnesium, assuming a 50 to 60 % bioavailability of organic magnesium (Coudray et al., 2005).

[00164] Assuming a three-fold higher bioavailability of calcium acetate when compared to calcium carbonate (Examples 6), the daily requirement of calcium from calcium acetate would be one third of that reported by (Bo-Linn et al., 1984) which is 250 mg of calcium from calcium acetate.

[00165] In this invention, magnesium was found to enhance calcium balance (Example 6). Therefore, it is necessary to have magnesium in the formula. If 250 mg of calcium in the form of calcium acetate is administered, a Ca/Mg ratio of 2/1 and 1/1 would provide 125 mg and 250 mg of magnesium, respectively. This would translate to 62.5 to 125 mg of absorbable magnesium, respectively, assuming a 50% bioavailability.

[00166] A5 plus vitamin D_3 has the best average in reducing the rate of bone loss in an OVX model (Figures 15 and 16). These results are consistent with that obtained in the balance study (Example 6). Calcium carbonate, as represented by Caltrate™ did not show any significant improvement over OVX control when the Distal Femur BMD was used for comparison (Figure 14).

[00167] Zinc has been shown to be essential for bone formation and the recommended daily allowance is 20 mg. It is found that zinc balance is dependent on nutritional status (Example 6). However, a positive zinc balance can be maintained if vitamin D_3 is incorporated into the formula.

[00168] Vitamin D_3 has been reported to increase the absorption of calcium from the gut; it also assists distribution of calcium into bone (Wasserman, 2004). We also found that vitamin D_3 is essential for calcium and zinc balance (Example 6). The recommended daily intake is 400 to 800 IU. This dosage is incorporated into the fortified extract.

[00169] It is also found that administration of A5 plus vitamin D_3 improved elemental balance of dietary calcium and magnesium (Example 6). This observation is significant because the less available form of calcium and magnesium was improved. The implication is that if a subject does not have enough elements in his diet, the supplementation of a low dose of the optimized formula plus that from the dietary source will provide adequate elemental daily requirements. Therefore, a lower dosage of A5 and vitamin D_3 can be used for maintaining bone health and possibly preventing osteoporosis.

[00170] Taking into account the solubility of the elements, its taste and convenience of administration, a two gram once a day dose of A5 (~220 mg calcium) plus vitamin D_3 will provide adequate amounts of elements and vitamin D_3 for the maintenance of bone health and prevention of osteoporosis.

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What is claimed is:

1. A composition comprising an initial composition of at least 22.75 percent calcium by weight extracted from calcium containing or synthetic calcium acetate-containing sources, wherein said initial composition is fortified with magnesium and zinc to provide a final composition of at least 4 percent by weight of calcium, at least 5 percent by weight of magnesium, at least 0.2 percent by weight of zinc, and at least 400 IU of vitamin D_3.

2. The composition of claim 1, wherein the calcium containing sources are pearls, corals, oysters, or natural mines.

3. The composition of claim 1 or 2, wherein the magnesium is in the form of acetate salt.

4. The composition of claim 1 or 2, wherein the zinc is in the form of acetate salt.

5. The composition of claim 1 or 2, wherein the composition comprises a weight ratio of calcium to magnesium of 0.5:1 to 4:1.

6. The composition of claim 1 or 2, wherein the composition comprises a weight ratio of calcium to magnesium of 1:1 to 2:1.

7. The composition of claim 1 or 2, wherein the composition comprises a weight ratio of zinc to calcium ranging from 0.05:1 to 0.20:1.

8. The composition of claim 1 or 2, wherein the composition comprises a weight ratio of zinc to calcium ranging from 0.05:1 to 0.1:1.

9. The composition of claim 1 or 2, wherein the composition comprises 5-40 mg of zinc per daily dose to be administered to a human adult.

10. The composition of claim 1 or 2, wherein the composition comprises 400 to 1200 IU of vitamin D_3 per daily dose to be administered to a human adult.

11. The composition of claim 1 or 2, wherein the composition comprises 50 to 500 mg of calcium and 25 to 500 mg of magnesium per daily dose to be administered to a human adult.

12. The composition of claim 1 or 2, wherein the composition comprises 100 to 300 mg of calcium and 50 to 150 mg of magnesium per daily dose to be administered to a human adult.

13. The composition of claim 1 or 2, wherein the composition comprises 400 to 1200 IU of vitamin D_3 per 220 mg of calcium.

14. The composition of claim 1 or 2, wherein said composition comprises more bioavailable calcium per unit weight than calcium carbonate.

15. A method of alleviating or preventing symptoms of osteoporosis in humans or animals, comprising the step of administering the composition of any one of claims 1 through 14 to said humans or animals.

16. The method of claim 15, wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult.

17. The method of claim 15, wherein the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

18. A method of increasing bone mineral density in humans or animals, comprising the step of administering the

composition of any one of claims 1 through 14 to said humans or animals.

19. The method of claim 18, wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult.

20. The method of claim 18, wherein the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

21. The use of any of the compositions recited in any one of claims 1 through 14 to alleviate symptoms of osteoporosis.

22. The use of claim 21, wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult.

23. The use of claim 21, wherein the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

24. The use of any of the compositions recited in any one of claims 1 through 14 to increase bone density.

25. The use of claim 24, wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult.

26. The use of claim 24, wherein the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

27. The use of any of the compositions recited in any one of claims 1 through 14 to facilitate dietary calcium and magnesium absorption.

28. The use of claim 27, wherein the composition comprises between 50 to 500 mg of calcium per daily dose to be administered to a human adult.

29. The use of claim 27, wherein the composition comprises between 100 to 300 mg of calcium per daily dose to be administered to a human adult.

FIGURE 1

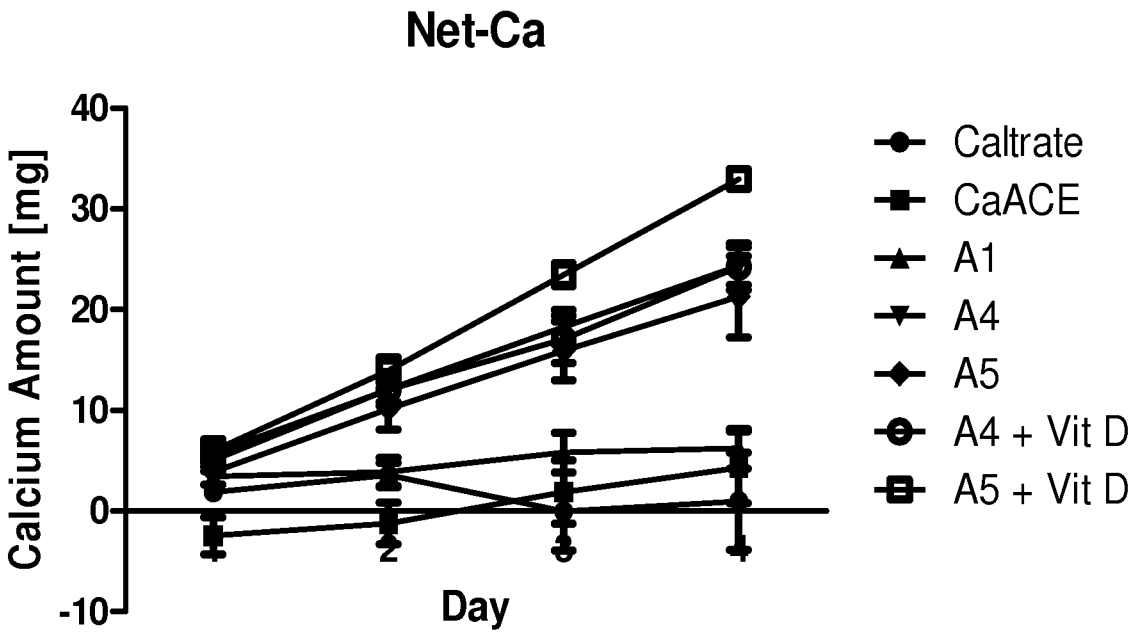

Net-Ca
Calcium Amount [mg]
40
30
20
10
0
-10
Day
Caltrate
CaACE
A1
A4
A5
A4 + Vit D
A5 + Vit D

FIGURE 2

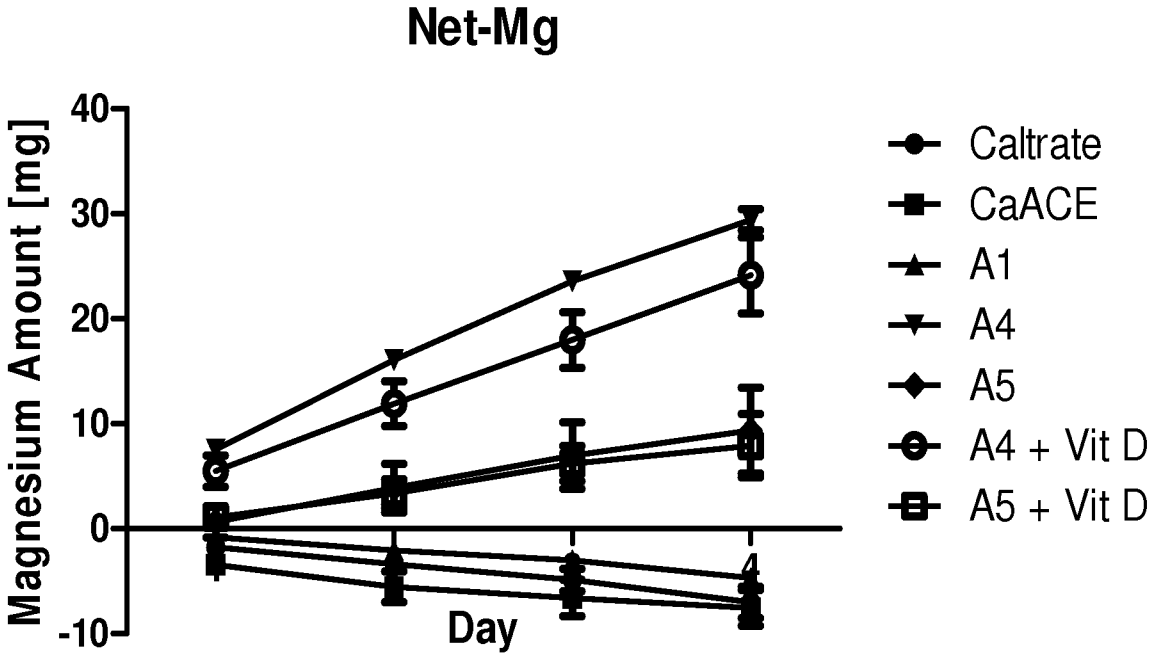

Net-Mg
Magnesium Amount [mg]
40
30
20
10
0
-10
Day
4
Caltrate
CaACE
A1
A4
A5
A4 + Vit D
A5 + Vit D

FIGURE 3

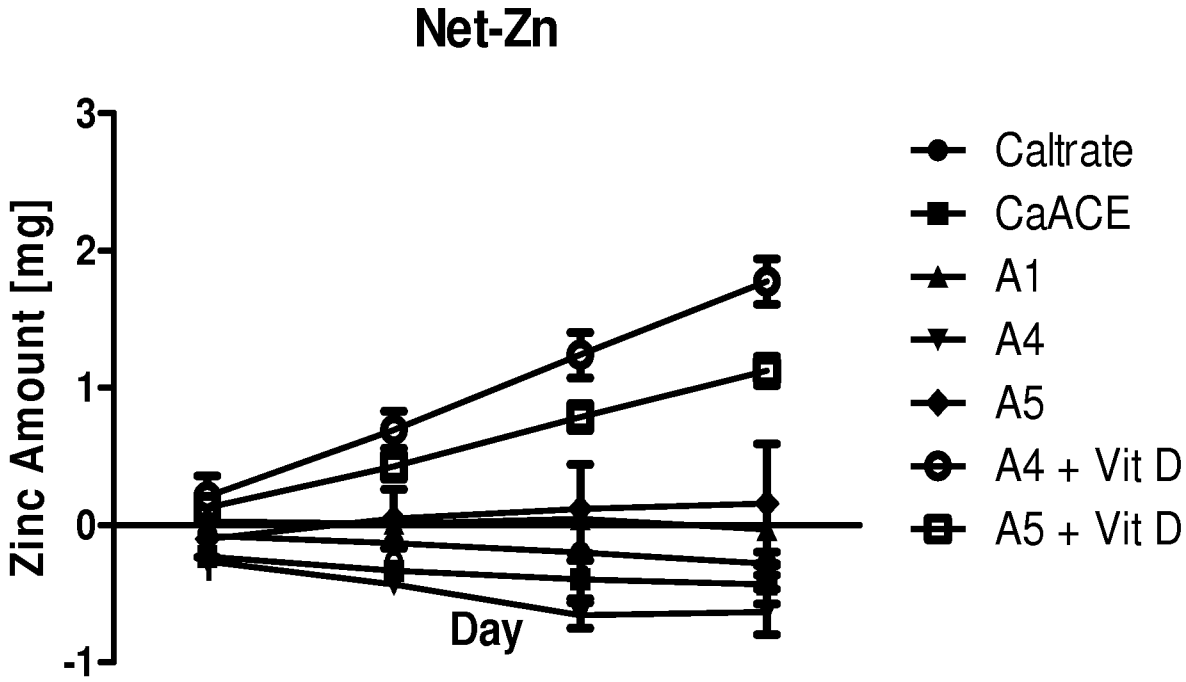
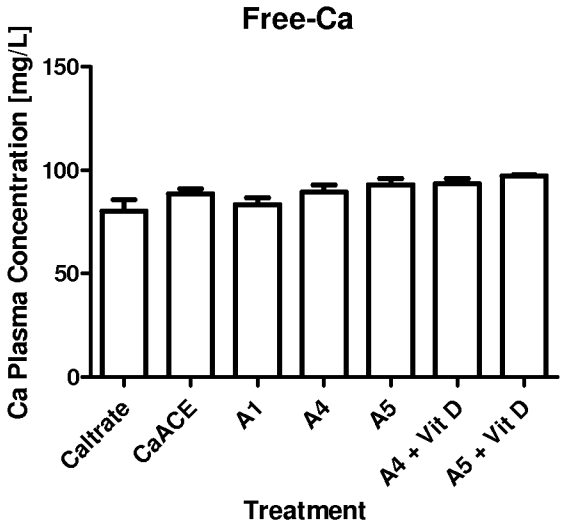
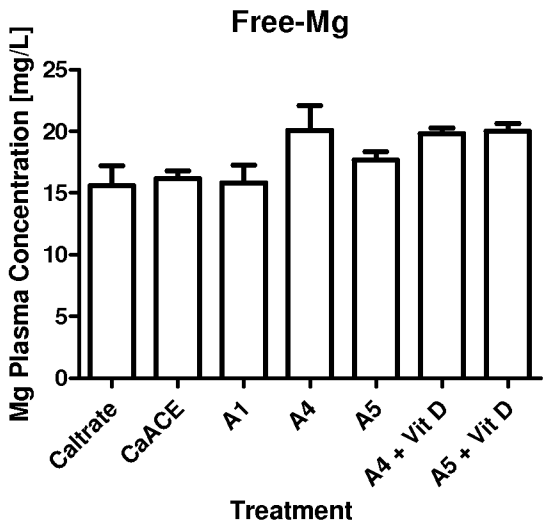

Net-Zn
Zinc Amount [mg]
3
2
1
0
-1
Day
Caltrate
CaACE
A1
A4
A5
A4 + Vit D
A5 + Vit D

FIGURE 4

A.


Free-Ca
Ca Plasma Concentration [mg/L]
150
100
50
0
Caltrate
CaACE
A1
A4
A5
A4 + Vit D
A5 + Vit D
Treatment

B.


Free-Mg
Mg Plasma Concentration [mg/L]
25
20
15
10
5
0
Caltrate
CaACE
A1
A4
A5
A4 + Vit D
A5 + Vit D
Treatment

C.

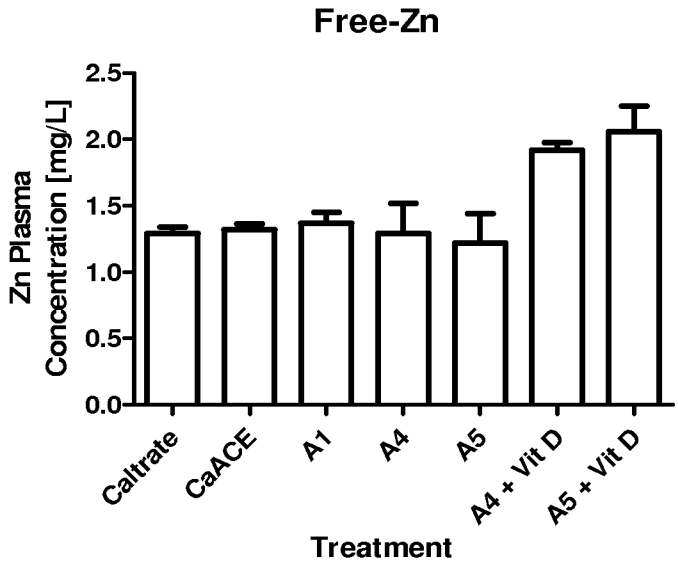

Free-Zn
Zn Plasma Concentration [mg/L]
2.5
2.0
1.5
1.0
0.5
0.0
Caltrate
CaACE
A1
A4
A5
A4 + Vit D
A5 + Vit D
Treatment

FIGURE 5

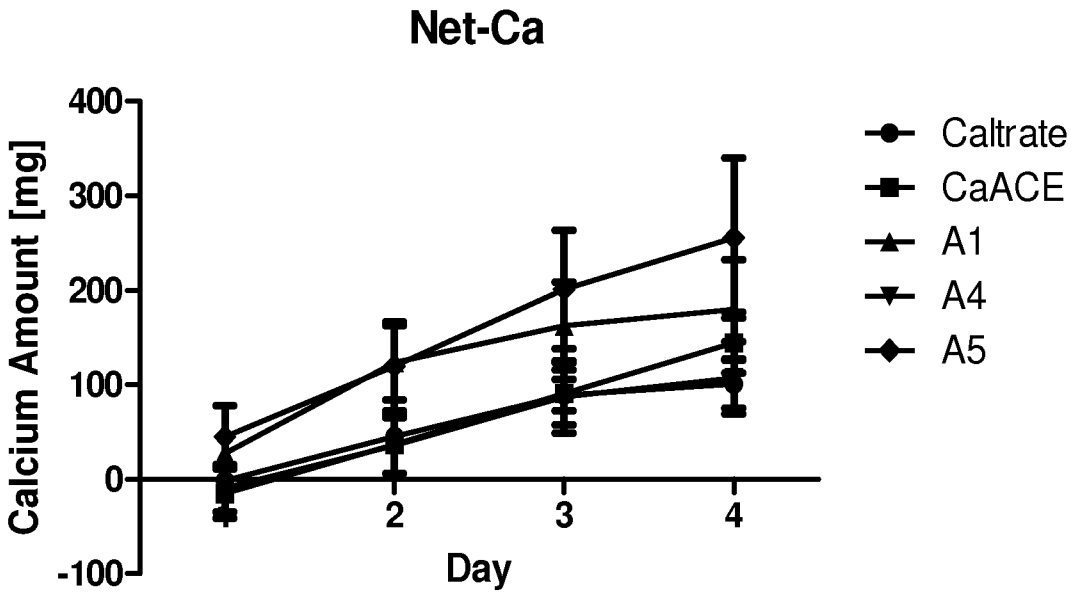

Net-Ca
Calcium Amount [mg]
400
300
200
100
0
-100
2
3
4
Day
Caltrate
CaACE
A1
A4
A5

FIGURE 6

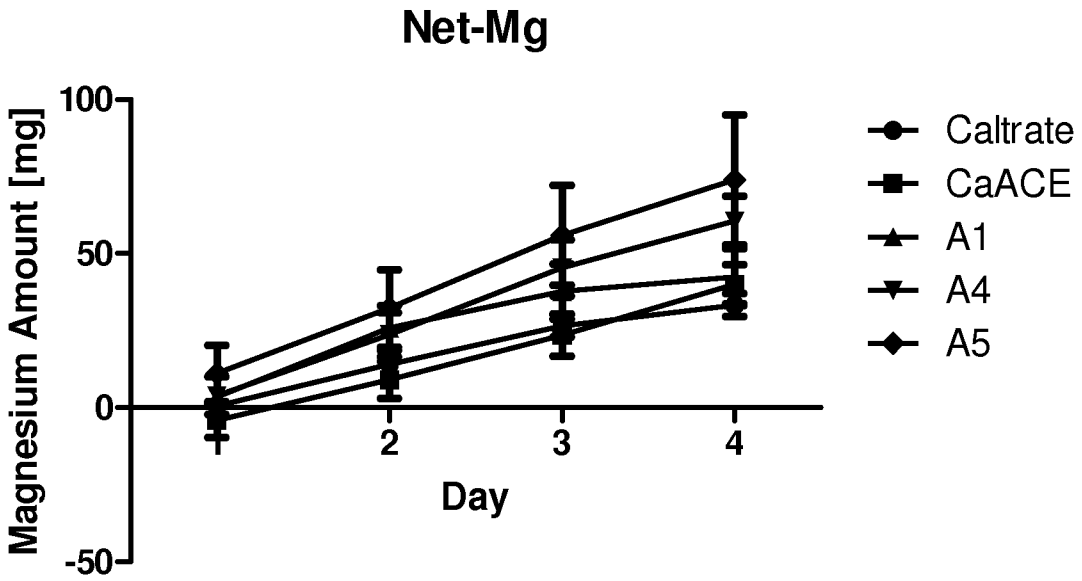
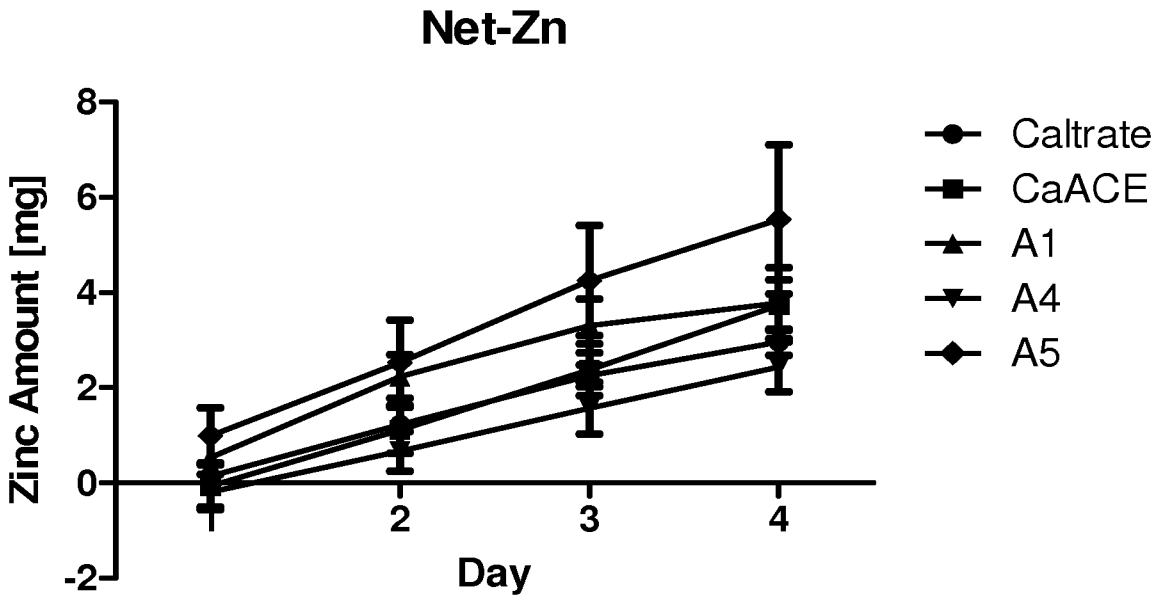

Net-Mg
Magnesium Amount [mg]
100
50
0
-50
2
3
4
Day
Caltrate
CaACE
A1
A4
A5

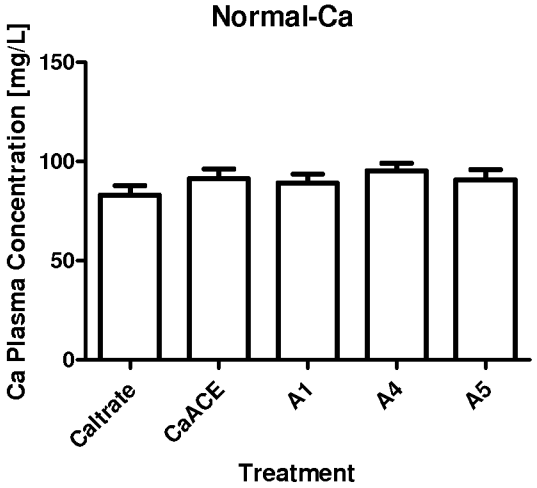
FIGURE 7



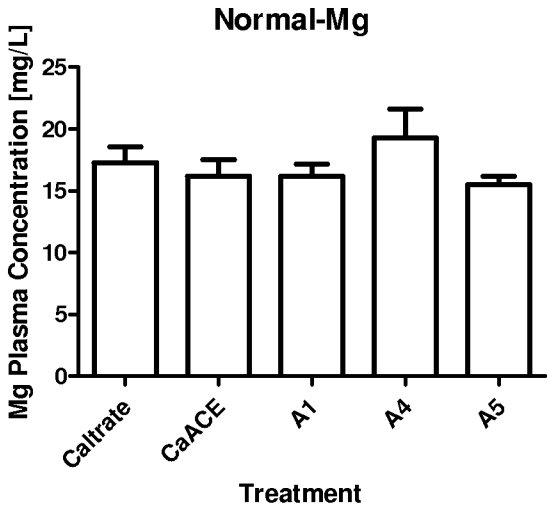
8/15

FIGURE 8

A.



B.



C.

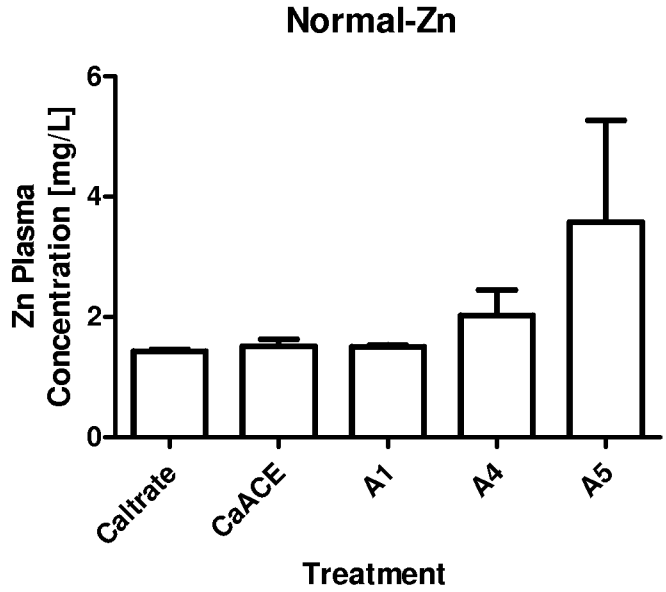


FIGURE 9

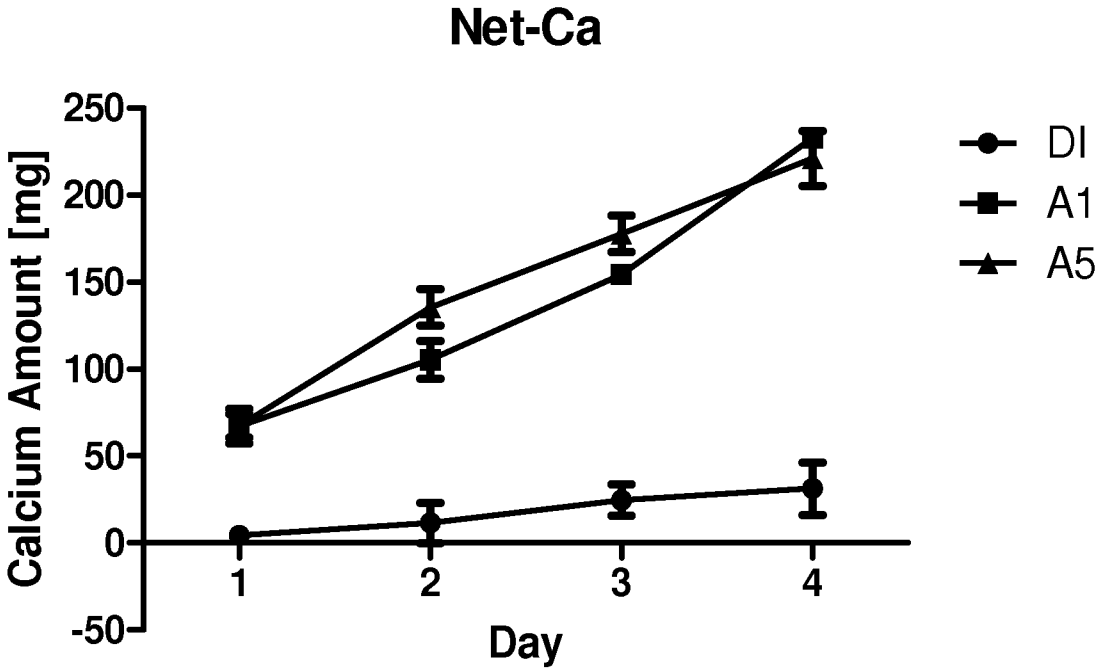

Net-Ca
Calcium Amount [mg]
250
200
150
100
50
0
-50
1
2
3
4
Day
DI
A1
A5

FIGURE 10

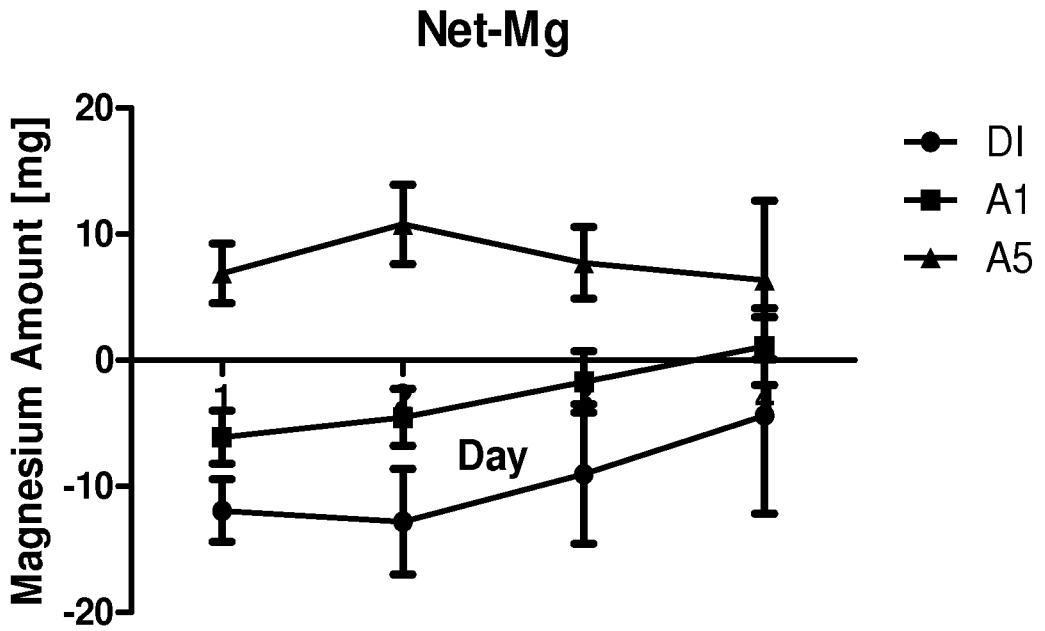


FIGURE 11

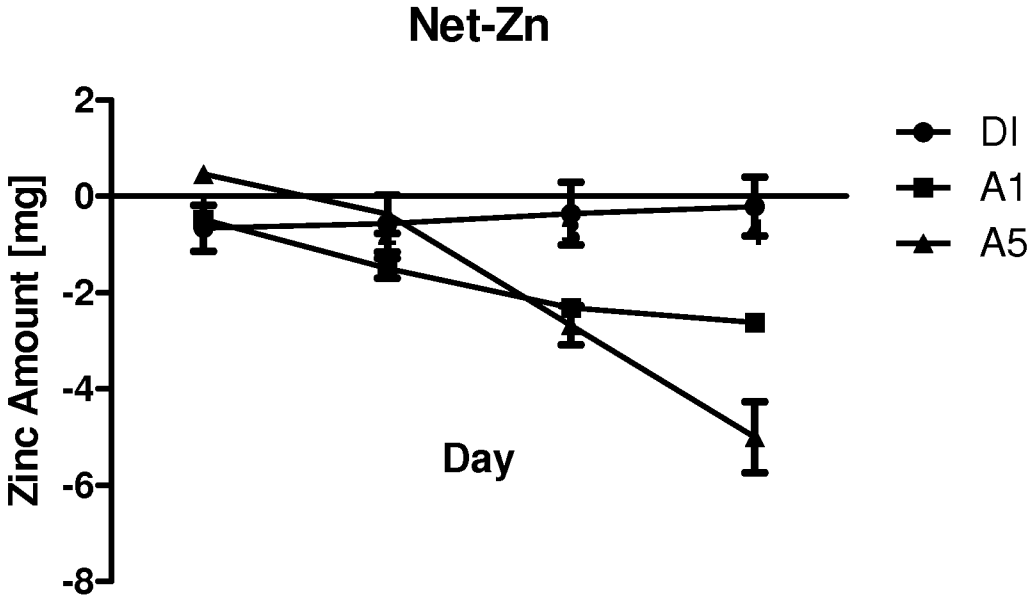
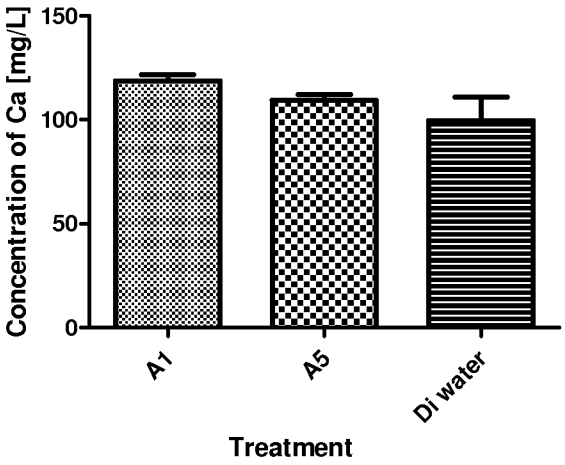
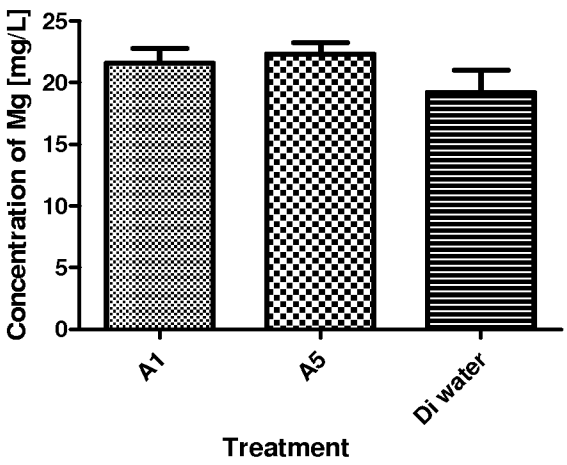


FIGURE 12

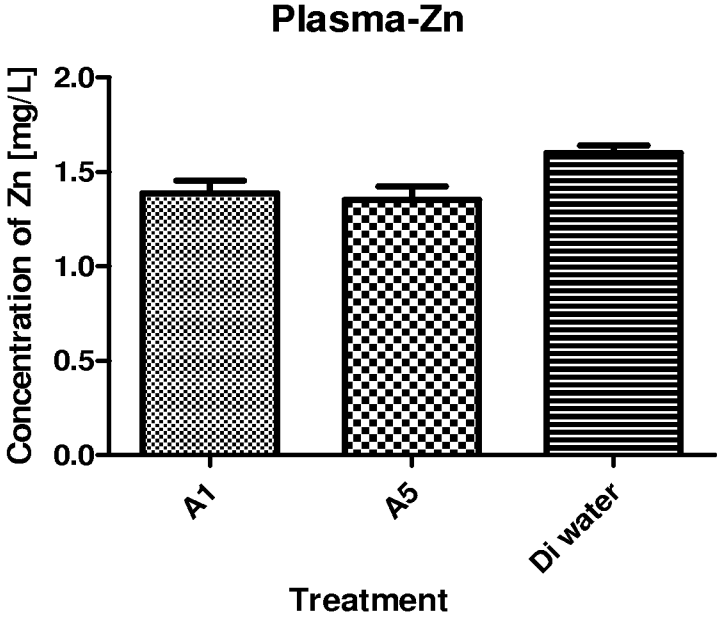
A.



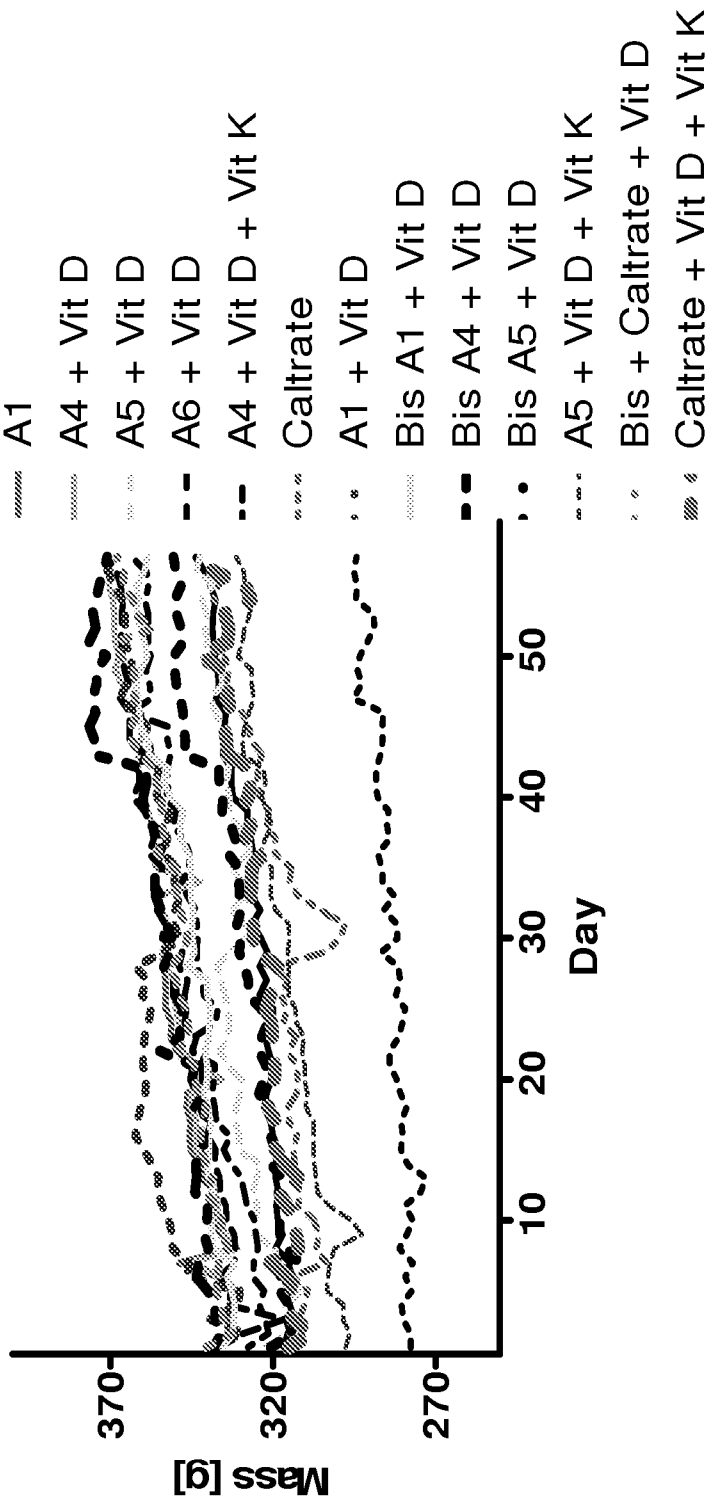
B.



C.

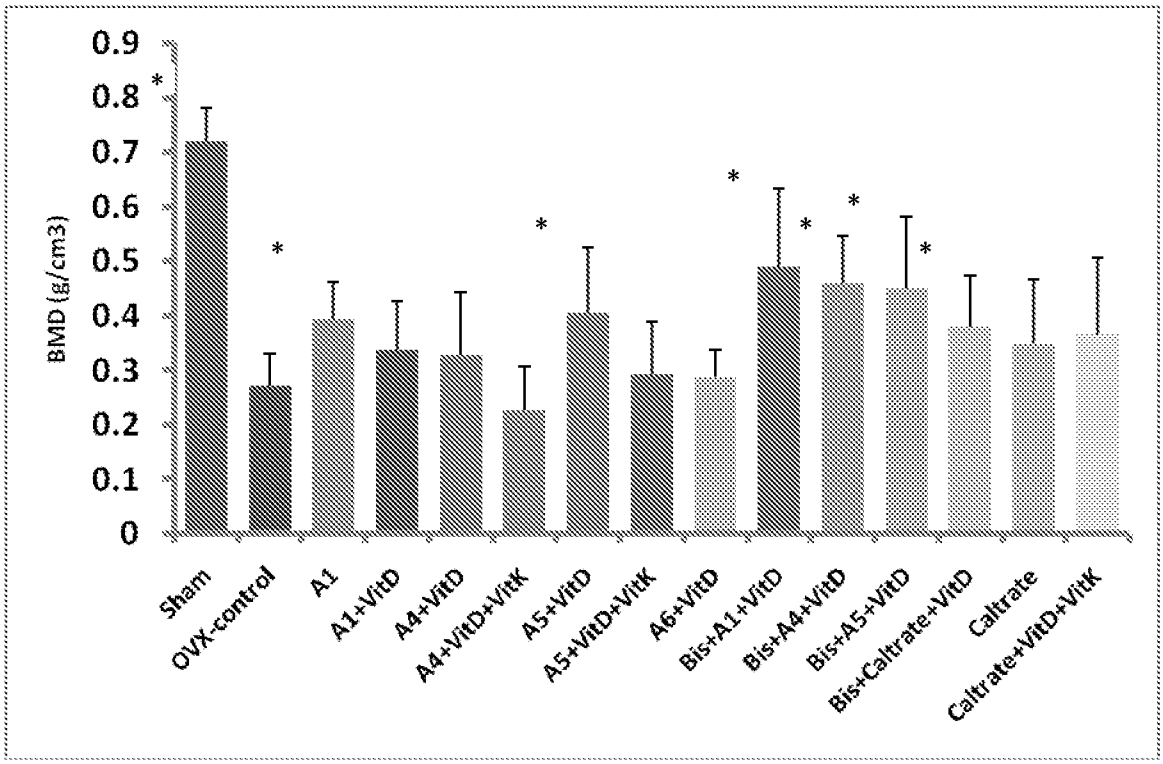


13/15
FIGURE 13



14/15

FIGURE 14



15/15
FIGURE 15