Preparation of the edible supplement product of calcium-D in form of tablet from powder of sepia skeleton (cuttlebone) and investigation of its physic-chemical properties

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Abstract

In vitamin D deficiency, calcium absorption from the intestines occurs, which increases the production of osteoclasts, which causes destruction of bones and ultimately osteoporosis. In this study, a tablet was made by use of cuttlebone spp., calcium and vitamin D, and its physicochemical properties such as powder flow ability, weight uniformity, friability, solubility and hardness were examined. The resultant outcomes showed that the prepared formulations have high level of potential, and after more accurate studies, it is possible to use the sepia skeleton to make tablets for the treatment of calcium and vitamin D deficiency related disorders.

Key words: osteoporosis, Cuttlebone, Tablet

Introduction

Osteoporosis is one of the most important metabolic diseases, especially in the elderly (1). Bone fractures are the most important side effects of the disease which causes great financial losses imposed on families and societies, in addition to illness and death in older people (2, 3).

Osteoporosis, a multifactorial pathology has been reviewed extensively; a review by Gaby about osteoporosis nutritional and hormonal management is an excellent and well referred source (4). Osteoporosis etiology evaluation in a special population can involve hormonal aspects, exercise patterns, nutrient intake, digestion and nutrient absorption (5).

The balance between the resorption and evolution is related with age and sex. Bone turnover takes place by a multitude of nutrients such as calcium, vitamin D and vitamin K. Adequate calcium intake is considered as one of the important nutritional factors to establish peak bone mass(6). Vitamin D which is necessary for optimal dietary Ca absorption should be sufficient as well (7).

Evidence supports the use of calcium, or calcium in combination with vitamin D supplementation, in the preventive treatment of osteoporosis in people aged 50 years or older. For best therapeutic effect, we suggest minimum doses of 1200 mg of calcium, and 800 IU

of vitamin D (for combined calcium plus vitamin D supplementation)(8).

The Sepia pharaonis is one of the enormous cuttlefish species, growing to 42 cm in mantle length and 5 kg in weight(9). The maximum recorded size for those males that have been raised in the laboratory is 16.2 cm, and for females 15.5 cm(10). It seems that Sepia pharaonis, at least, is a complex of three species, Sepia pharaonis I, commonly located in the Red Sea and Persian Gulf, S. pharaonis II, which lives in Japan to the Thailand Gulf and northern Australia; and S. pharaonis III, which lives in the Indian Ocean to the Andaman Sea (11).

However, natural compounds such as natural dietary fibre (12), herbal extracts (13), natural clinoptilolite (14) and medicinal plants, spices, vegetables and crude drugs (15, 16) have been used as antacid drugs.

Since cuttlebone (CB) is a natural compound with a high percentage of CaCO3, it can be used and formulated by different fillers as a marine natural anti-Osteoporosis drug.

The purpose of this study was to evaluate the dietary supplement of calcium-D in the form of tablets from the cuttlebone of the Sepia skeletal system, in order to help absorb calcium better.

Material and methods

Cuttlebone was gathered from Bushehr coast, then was washed and dried in free air in order to lose its smell. After drying, the clean cuttlebone was powdered and completely mixed to 60-100 mesh size. Then CaCO3 components were measured and the metal elemental analysis was determined by mechanical methods. Tablets were then prepared using a single-tube. A tablet with a direct injection of 500 mg CB of vitamin D (200 units) and magnesium stearate lubricant produced, and its properties such as powder flow ability, friability, strength, and disintegration time were studied and compared.

Analysis of elements and compounds of CB were characterized by means of CHN, XRF, XRD and FTIR techniques. Data of XRD and XRF techniques was from a previous study (GP-94160) (17, 18).

Powder flow ability, friability, weight uniformity, hardness, disintegration time and dissolution test for the formulated drug and marketed dosage forms were measured according to USP.

Flow ability of CB Powder

Flow ability of CB Powder with fillers was measured by flow meter apparatus in g/sec. For all of the formulations, the corresponding powders were poured into the funnel of apparatus and flow ability of powders was calculated by apparatus.

Friability test

Friability Tests were evaluated with friability apparatus for 10 weighted tablets of formulated drugs, with 25 rpm for 4 minutes. Friability percent was calculated via the following formula:

Friability (%) = (W1-W2)/W1×100

None of the tablets should not be beaked or capped. Friability percent should be less from 0.5-1 %.

Weight uniformity

10 tablets from each formulation were selected accidentally and weighed by digital balance.

Hardness Test

Hardness of formulated drugs was measured by hardness apparatus in Kg. In this method 10 drugs were selected accidentally from each formulated drugs and hardness of them was determined by apparatus. The favoured hardness should be obtained between 4-6 Kg.

Disintegration Test

Disintegration time for formulated drugs was measured by related apparatus in distilled water environment. For this purpose, 6 tablets from formulated drugs were selected and placed into the tubes of the apparatus in water bath 37°C with regular movements.

Disintegration time of the first and last tablets were determined.

Dissolution

Three tablets of sepia and three standard tablets were selected randomly and each tablet was placed into a beaker containing 50 ml of phosphate buffer (pH= 7.4), and three drops of ethanol were added to beaker in order to obtain better dissolution of the tablets. The beaker was placed on a heater stirrer at 37 ° C. At time intervals of 5, 10, 20, 30 and 45 minutes (determined by the calibration curve of Vit. D3), 2 ml sample from each beaker was separated, and in order to keep the content invariable, 2ml buffer was added to the solution. According to data of USP, the spectrophotometer was adjusted at a wavelength of 284 nm for measuring the concentration of vitamin D3 and then absorption of samples were measured at this wavelength for determination of vitamin D3 concentration. Vitamin D3 was used as a standard solution in dissolution test.

Results

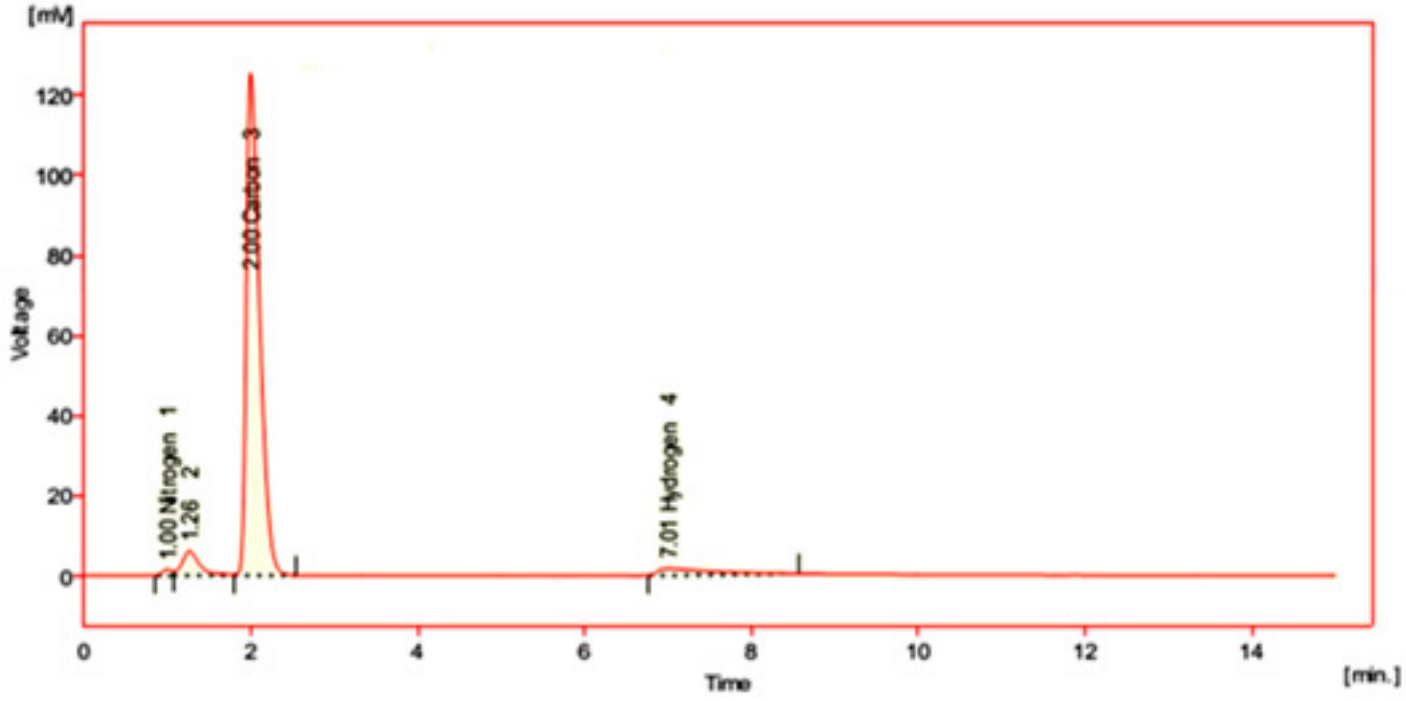
A. Elemental and chemical analysis of Crude CB

CHN analysis of CB is showed in Figure 1 and Table 1.

Table 1: CHN analysis of Crude CB

	Reten. Time [min]	Response	Weight [mg]	Weight [%]	Peak Type	Element Name
1	0.997	10.732	0.007	0.37	Refer	Nitrogen
3	1.997	1372.977	0.231	12.29	Refer	Carbon
4	7.007	75.378	0.025	1.34	Refer	Hydrogen
	Total		1.879	14		

Figure 1: CHN analysis of crude CB



	Reten. Time [min]	Response	Weight [mg]	Weight [%]	Peak Type	Element Name
1	0.997	11.031	0.007	0.37	Refer	Nitrogen
3	1.997	1372.977	0.231	12.29	Refer	Carbon
4	7.007	75.378	0.025	1.34	Refer	Hydrogen
	Total		1.879	14.00		

CHN analysis of CB showed the amount of C 12.29%, N 0.37% and H 1.34%. The amount of C is more than two other elements (Figure 1), because CB is composed mainly of CaCO3 and 12.29% of C is comparable to 12% of C in CaCO3. According to average of measured CaCO3 that was obtained 92.08%, from 1.879 mg of CB for CHN analysis, 1.73 mg CaCO3 is calculated whereby 12% of it is 0.2076 mg C or 11.05% C. This amount is well comparable to 0.231 mg C or 12.29% C in Table 1. The higher amount of C is related to C of chitin and chitosan of CB.

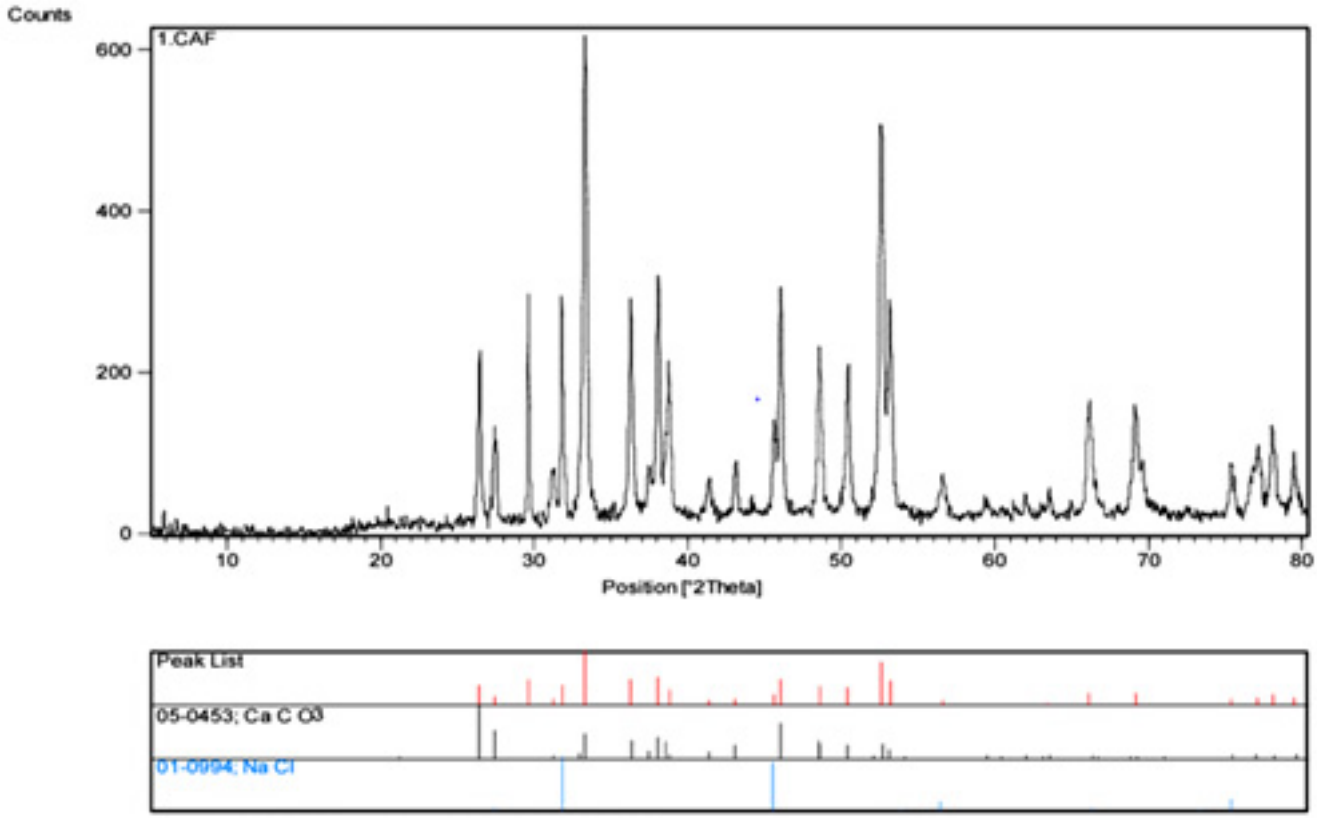
XRF analysis of CB showed existence of the following elements such as Na, Mg, K, Si ,S, P, Cl and specially Ca.

XRF analysis of CB is shown as 44.71% CaO (or 31.93% Ca). In comparison to the average of carbonate based on Ca (92.08%) that measured in CB (or 36.83% Ca), so the amount of Ca is well determined (Table 2).

Table 2: XRF analysis of crude CB

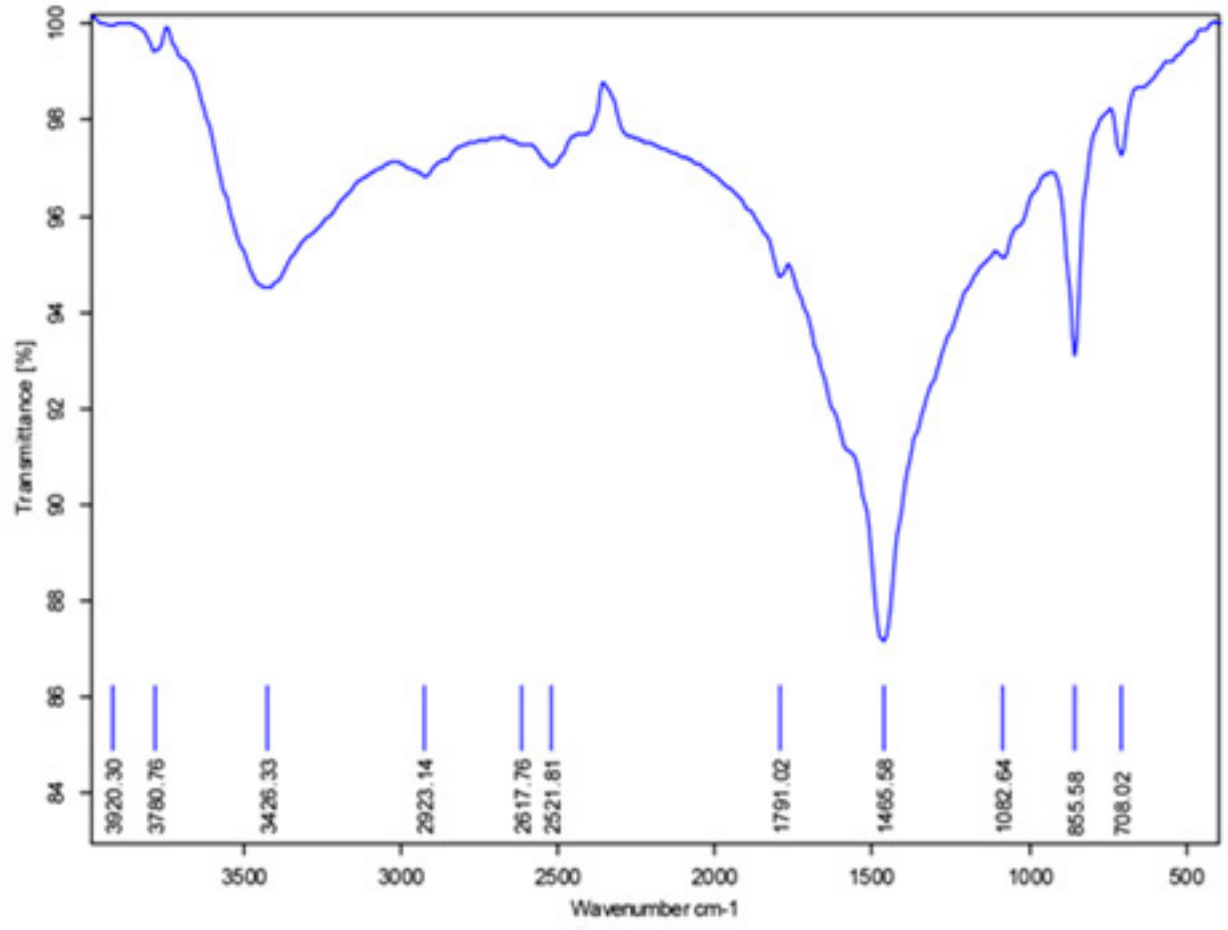
MgO (%)	K2O (%)	Na2O (%)	CaO (%)	Fe2O3 (%)	Al2O3 (%)	SiO2 (%)
0.36	0.07	2.25	44.71	0.03	0.04	0.12
Sr (ppm)	**Cl (ppm)**	**L.O.I (%)**	**SO3 (%)**	**P2O5 (%)**	**MnO (%)**	**TiO2 (%)**
1756	24500	53.96	0.255	0.102	0.006	0.012

Figure 2. XRD spectrum of CB powder



XRD spectrum of CB confirmed the presence of CaCO3 (Figure 2).

Figure 3. FTIR spectrum of CB



FTIR spectrum of CB showed the following absorption areas.

Peak at 3426 cm-1 is related to OH and NH2 bonds in chitin. Peaks at 1465, 855 and 708 cm-1 are attributed to C-O bond in carbonate ion. Absorptions at 2521 cm-1 and 2923 cm-1 are related to HCO3- ion and C-H bonds, respectively (Figure 3).

B. Review the properties of the tablet

The average weight of the tablets in the laboratory and commercial tablets available in the market was in normal range, and the average of the tablets prepared in the laboratory were lighter than the tablet samples in the market.

The results of friability testing of CB prepared tablets in the laboratory and standard tablets in the market are shown in Table 3.

In Table 3, W1 (10 pounds before friability testing) and W2 (weight of tablets after friability testing) are also included.

Table 3: Friability percentage of tablets in the market and tablets in the laboratory

friability	W_2	W_1	Tablet
0.19	6.516	6.529	Tablet prepared in the laboratory
0.37	13.49	13.54	Tablet prepared from those available in the market place.

According to the results, the friability percentage of all prepared tablets was less than 1, so the result of the friability test was desirable.

The results show that the hardness of the formulation prepared in the laboratory is 7.78 pounds. Also, the hardness of the tablets in the market is calculated to be 13.68 pounds. consequently, the hardness of the sepia tablets was less than standard tablets in the market. (Table 4).

Table 4: Hardness and strength of tablets prepared from CB and tablets available on the market

Hardness (kp)	Tablet prepared from the market	Hardness (kp)	Tablet prepared in the laboratory
13.5	1	5.6	1
13.2	2	9	2
14.8	3	7	3
15	4	7.9	4
13	5	6.7	5
15	6	8.2	6
12.1	7	6.3	7
12.5	8	8	8
13.9	9	7.7	9
13.5	10	11.4	10

The results of the disintegration test for tablets prepared from CB in formulations and standard tablets in the market are shown in Table 5. The disintegration time for the first and the last tablet is presented in Table 5.

Between the tablets provided in the laboratory and standard tablets, the first and the least disintegration time was for the tablets in the market; and the last disintegration was for the tablets prepared in the laboratory.

The disintegration time of the sepia tablet is short, so it is desirable to test its disintegration.

Table 5: Disintegration time of the tablets in the laboratory and in the market

Disintegration time (seconds)	tablets in the market	Disintegration time (seconds)	tablets in the lab	
18.3	1	24.11	1	The first disintegration
19.11	2	25.25	2	
21	3	25.48	3	
22.38	4	26.02	4	
24.14	5	27.01	5	
24.5	6	27.37	6	The last disintegration

The results of the dissolution test for CB-prepared tablets in the formulation and tablets in the market are shown in Table 6 and Table 7, respectively.

The results show that the dissolution rate of CB tablets is higher than the standard tablets in the market; and according to the table and sepia tablet read absorptions, it is determined that about 85% of the drug is released in 45 minutes (Q = 85%).

Table 6: Results of the dissolution test for standard tablets in the market

Number 3 absorption	Number 2 absorption	Number 1 absorption	tablets in the market / Time (min)
0.198	0.207	0.135	5
0.237	0.247	0.205	10
0.268	0.270	0.261	20
0.360	0.324	0.307	30
0.390	0.346	0.406	45

Table 7: Results of the dissolution test of CB prepared tablets

Number 3 absorption	Number 2 absorption	Number 1 absorption	CB tablet / Time (min)
0.182	0.183	0.190	5
0.225	0.231	0.210	10
0.264	0.270	0.284	20
0.335	0.319	0.317	30
0.367	0.375	0.337	45

Absorption of various concentrations of Vitamin D3, which was measured by the spectrophotometer, is given in Table 8 and Figure 4. As the results indicate, ascending absorption increases.

Table 8: Absorption of various concentrations of Vitamin D3

5 mg/ml	4 mg/ml	3 mg/ml	2 mg/ml	Different concentrations of Vitamin D3
0.44	0.357	0.253	0.182	Absorb

Figure 4: Diagram of absorption/ concentration of vitamin D3

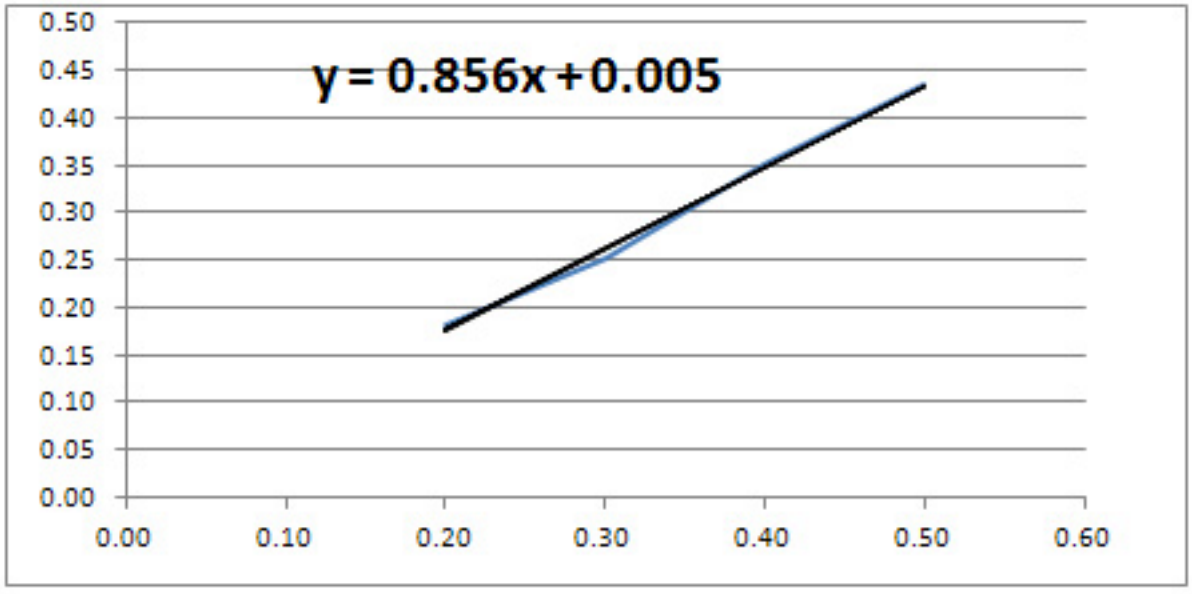


Figure 5: Diagram of concentration/ time of standard tablet

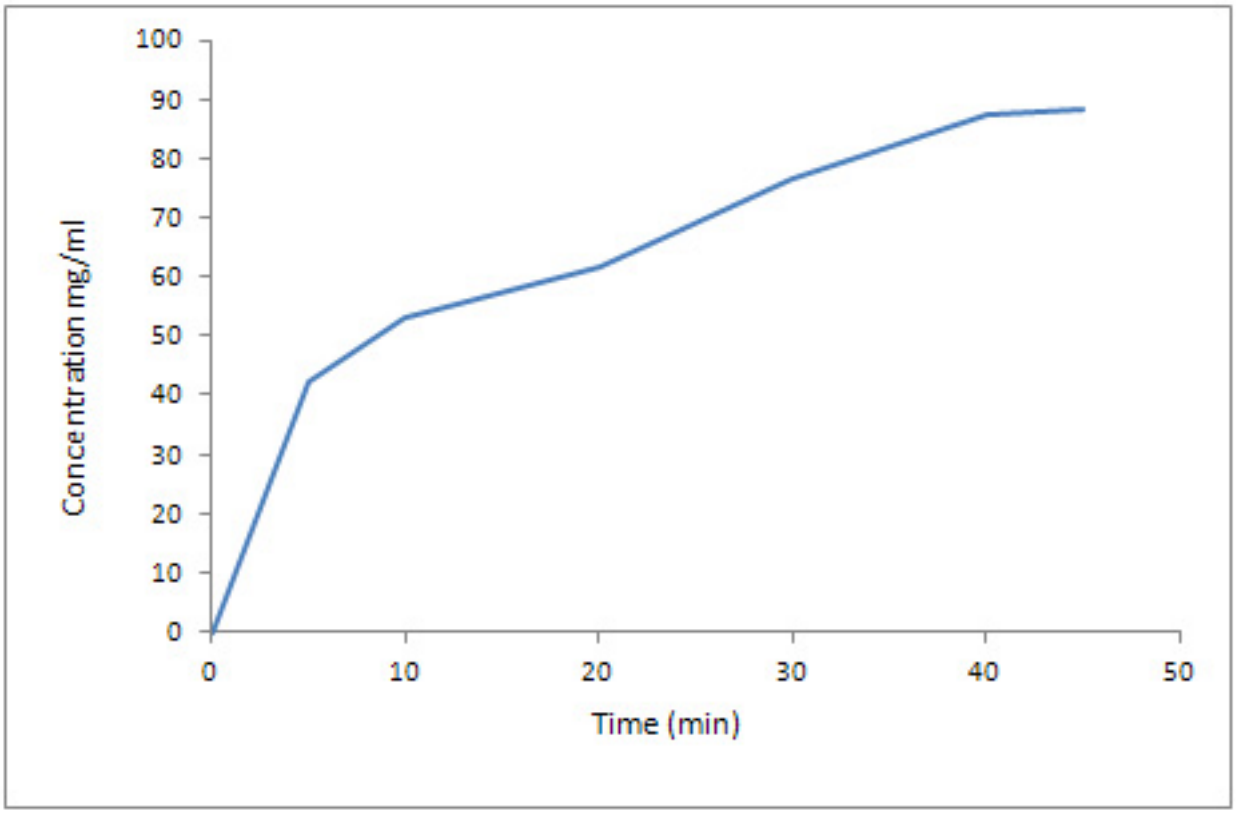
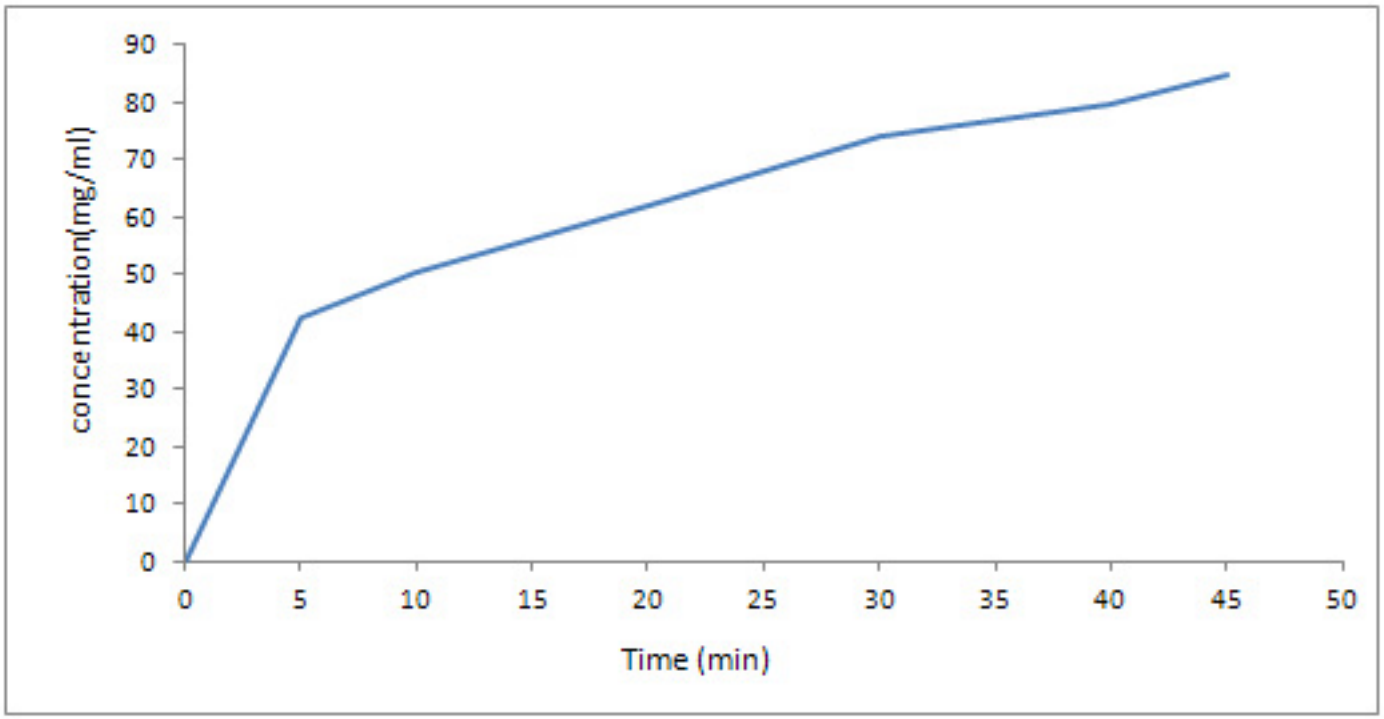


Figure 6: Diagram of concentration/ time of CB prepared tablet



Discussion

Nowadays, side effects, drug interaction and other problems of chemical drugs have caused increase in use of natural or herbal drugs in treatment of disease. Moreover, one of the important considerations is the cost. One of the natural marine compounds that has Osteoporosis property is cuttlebone (CB).

In this research, we collected sepia fish and tried to produce a dietary supplement of calcium-D. This dietary supplement shows a better result in osteoporosis treatment and, in addition, reduces the side effects of the drug.

The importance of disintegration time of tablets is well known in the bioavailability of drugs and drug release. In fact, in order to be able to absorb the drug from the tablet form, first the tablet should be disintegrated and then the drug will be released. Therefore, proper disintegration of the tablet is important. The formulation of a product has a significant effect on the rate of disintegration and dissolution. Dissolvents open the tablets with different

mechanisms. These mechanisms include the effect of conjugation, swelling, hydration, change in volume or position and release of gas (19)

In this investigation, the weight of all formulations was uniform and also had a good degree of hardness and low friability percentage. As mentioned in various studies, the changes in formulation weights are less than ± 5%, the friability rate is less than 1% and the hardness is between 4 and 6 within the standard pharmaceutical range (20, 21).

Some researchers have reported that the lower hardness in direct compression is due to increased friability of prepared tablets (22, 23). However, in the present study, due to appropriate CB structure and prepared formulation, all formulations had a good hardness and friability.

Finally, according to the obtained results in this study and the desirable properties of the prepared formulations such as hardness, dissolution, friability, weight uniformity and disintegration time of the tablet, the prepared formulation has a high potential and can be studied more precisely. The Septal Skeleton was used to make tablets to treat calcium and vitamin D deficiency. Also, due to the nature of the marine environment and much less side effects, CBs, if confirmed by the results of the study, can be used to replace tablets in the market due to their high performance.

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