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Use of Eggshells as a Raw Material for Production of Calcium Preparations

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Abstract

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The kinetics of calcium release from tablets obtained from modified eggshells in the form of calcium citrate and calcium carbonate was investigated. Calcium release showed the first-order kinetics. After 30 min of the experiment, 79.93% of calcium was released from tablets obtained from modified eggshells in the form of calcium citrate, reaching ~100% after 3 hours. For tablets produced with calcium carbonate, these values were 7 and 60%, respectively. The half-time of calcium release from tablets containing calcium citrate was $t_{50\%}$ = 0.5 h and for tablets containing calcium carbonate it was $t_{50\%}$ = 2.2 h, so calcium in the form of calcium citrate was released 4 times faster. These results can be connected with different solubility of calcium salts. The hardness of tablets with calcium carbonate was by 30 N lower than the hardness of tablets with calcium citrate. It is associated with particular physicochemical properties of calcium salt. Calcium citrate can exist in several states of hydration while calcium carbonate is anhydrous. These properties have an influence on the hardness of tablets.

Keywords: calcium release; calcium carbonate; calcium citrate

Calcium preparations contain carbonate, citrate, or gluconate salts which are not always effective (Dolińska *et al*. 2008a; Ueda & Taira 2013). Because of that, natural sources of minerals and vitamins are becoming more popular (Schaafsma & Pakan 2000; Oliveira *et al*. 2012). Eggshells can be alternatively used as a natural source of calcium and are characterised by higher solubility when compared to currently used oyster shells (Szeleszczuk *et al*. 2015). The eggshell consists of 95% of calcium carbonate, 3.5% of glycoproteins, and proteoglycans (Cordeiro & Hincke 2011). The inner shell membrane contains glucosamine, chondroitin sulphate, hyaluronic acid, type I collagen, and a high amount of proteins and microelements such as magnesium, strontium, zinc, barium, fluorine, which could have positive effects on bone metabolism (Ruff *et al.* 2012). It has been noted that the powder from eggshells has desirable properties, such as easy ionisation at low stomach pH and high calcium content (36–39%) (Cordeiro & Hincke 2011; Ruff *et al*. 2012). It was also proved that the natural powder of inner shell membranes significantly decreases joint stiffness, reduces pain and inflammatory condition in patients with osteoarthritis (Ruff *et al*. 2009). 37% of eggshell calcium is in the form of carbonate with low bioavailability for the organism. According to these reports, a new technology of eggshell processing was developed. Eggshells were roasted in the presence of citric acid (Ryszka *et al*. 2007, 2014).

The aim of this study was to determine the *in vitro* availability of calcium from tablets containing calcium carbonate (synthetic raw material) or calcium citrate obtained from chicken eggshells (natural raw material).

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MATERIAL AND METHODS

Reagents. Calcium citrate was obtained from eggshells (FZNP Biochefa; Ryszka *et al*. 2007, 2014), calcium carbonate (POCH), inulin (Brenntag, Kędzierzyn-Koźle, Poland), potato starch (PEPEES), and magnesium stearate (Chem&Pol, Warsaw, Poland) were analytically pure and complied with quality standards.

Tablets with synthetic calcium carbonate were produced from calcium carbonate which was wet granulated with inulin syrup. Obtained granulate was dried at 60°C/24 hours. Next, magnesium stearate was added and that kind of mass was tabletted.

Tablets with calcium citrate were produced from eggshells with its membranes (Ovopol, Nowa Sól, Poland). Eggshells were mixed with citric acid and roasted at 120°C/2 h (Ryszka *et al*. 2007, 2014). Obtained granulate was mixed with other ingredients and tabletted.

Tabletting was performed with a rotatory tablet press with 30 matrixes and 12 mm spherical stamps (Fette, Schwarzenbek, Germany). The composition of tablets is presented in Table 1.

Physicochemical properties of tablets. Obtained tablets were investigated for physicochemical properties according to the Polish Pharmacopoeia (FP X 2014). Mean mass (mg), calcium content (mg), friability (%), hardness (N), disintegration time (min), and pharmaceutical availability (%) were determined for both preparations.

To determine the amount of calcium(II) ions a validated spectrophotometric method was used (Calcium O-CPC Kit; Pointe Scientific, Canton, USA). It is based on the reaction of calcium ions with *o*-cresolphthalein complexone (CPC) in the alkaline environment. The intensity of colour was measured with a UV-VIS 'Marcel Media' spectrophotometer (Marcel, Zielonka, Poland) in 1.0 cm glass cuvettes at a wavelength of λ = 570 nm. The photometric accuracy of the spectrophotometer was ± 0.005 A.

Table 1. The composition of tablets with calcium carbonate or calcium citrate

Composition (mg)	Tablets with calcium	
	carbonate	citrate
Salt	250	430
Inulin	87	200
Potato starch	–	150
Magnesium stearate	3	20

The empirical regression equation $y = 0.0585x - 0.0001$ was used to establish the relationship between calcium ion content and absorbance. The significance of the equation was $R^2 = 0.9974$; $P < 0.01$ and linearity up to 20 mg/dl.

Friability. 20 tablets are weighed and rotated in the drum of a tablet friability test apparatus (Erweka**,** Heusenstamm, Germany) for 4 min (25 revolutions/min). The difference in weight indicates the rate of friability (%).

Hardness. The test was conducted with a MultiTest 50 tablet hardness tester (Sotax, Thun, Swiss). The force is applied to the tablet until it breaks and this value is measured. Obtained results were presented as an average force value expressed in newtons.

Disintegration time was determined in 500 ml of 0.1 M HCl at 37°C with the use of MRT 1a (Polfa, Kraków, Poland). Tablets were placed separately in tubes which were limited from the bottom with a sieve and burdened from the top with cylindrical rings. The tube with the tablet was moved up and down through the distance of 5.5 cm at a frequency of 30 cycles per minute.

The speed of calcium release from 10 tablets was measured on a DT 600 paddle apparatus (Erweka, Germany) for 5 h (37°C, 75 rpm) using 900 ml of artificial gastric juice (0.1 M/l hydrochloric acid, pH = 1.2). The samples in the amount of 5 ml were collected every 30 min and filtered through the filter (0.45 μm pores) and then mixed with 5 ml of artificial gastric juice. The amount of released calcium in the collected samples was determined.

Based on the obtained results, it was determined that calcium release showed the first-order kinetics. The parameters of this process such as calcium release rate constant (k) and half-time of calcium release ($t_{50\%}$) were determined. The calcium release rate constant was calculated according to the following equation:

$$k = \ln C_1 - \ln C_2 / t_2 - t_1 \quad (h^{-1})$$

where: C_1, C_2– calcium concentration at time t_1 or t_2

The half-time of calcium release was calculated according to the equation:

$$t_{50\%} = 0.693/k \quad (h)$$

Statistical analysis. The percentage of released calcium in the unit of time was determined and profiles of calcium release were plotted. The results were calculated as mean values (± SD). The statistical

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analysis was carried out using Microsoft Excel and Statistica for Windows 5.1 (StatSoft Poland Sp. z o.o., 1997) software: Pharmaceutical Analysis, Drug Release Profile. Release profiles were compared using the Weibull distribution methodology with $P < 0.05$. Student's t-test was used to establish statistical significance with $P < 0.05$.

RESULTS AND DISCUSSION

Obtaining calcium citrate from chicken eggshells had several important goals. Firstly, it was the elimination of microbial contamination of raw materials. The consumption of uncooked eggs and eggshells may result in *Salmonella enteritidis* infection. Studies show that the powder from the eggshells not treated with any bacteria-inactivating agents (such as heat or microwaves) is affected by the increased bacterial growth of raw material (up to 90×10^5 CFU/g) (Hassan 2015). Our own synthesis conducted under a certain temperature (120°C/2 h) effectively inhibited the growth of bacteria and provided adequate sterility of the raw material (Ryszka *et al*. 2007, 2014). Secondly, there was a difference in the availability between calcium citrate and calcium carbonate. Numerous clinical studies have shown that calcium citrate has greater availability than calcium carbonate (Reginster *et al*. 1993). This is undoubtedly related to the solubility of these salts. Calcium carbonate is soluble practically only in a strongly acidic medium, and calcium citrate is well soluble in neutral and alkaline media, which affects the availability of the latter salt especially in the upper sections of the small intestine (for example in the duodenum, pH = 7). It is also worth mentioning that calcium is absorbed in the alimentary tract in ionised form and a higher dissociation constant of calcium citrate compared to the carbonate may explain the greater bioavailability of the salt (Hansen *et al*. 1996). An additional advantage of calcium citrate compared to calcium carbonate is that the carbonate is poorly absorbed in patients with stomach hypoacidity. In this disease calcium intake in the form of a citrate salt is recommended (Dolińska *et al*. 2008b). Calcium supplements are generally well tolerated and do not have much effect on the absorption of other microelements. Occasionally occurring side-effects such as constipation or flatulence can be removed by replacing preparations containing calcium carbonate with preparations containing calcium citrate (Sanders *et al*. 2009).

The characteristics of obtained tablets are presented in Table 2. The calcium content in these tablets was 100 mg. The friability of the tablets was consistent with FP X. The hardness of tablets with calcium carbonate was by 30 N lower than the hardness of tablets with calcium citrate. Obtained calcium citrate may be characterised by different amount of water of hydration. Hydrated salts are characterised by completely different physicochemical and mechanical properties from those of non-hydrated salts. Because of diverse stability among all hydrates dehydration may occur during production, modification, or storage (Sakata *et al*. 2005) and it may affect the hardness of tablets. The disintegration time of tablets was 4 min for tablets with calcium carbonate and 13 min for tablets with calcium citrate, which complies with requirements for orally administered preparations. The time within which the tablet disintegrates or dissolves is one of the parameters indicating pharmaceutical bioavailability of a substance. The speed of substance release indicates the speed of substance absorption into the bloodstream. Figure 1 presents calcium release profiles from tablets with calcium citrate and tablets with calcium carbonate from 0 to 5 hours. The comparison of release profiles was made by the Weibull method and showed statistical significant differences at $P < 0.05$. The speed of calcium release from tablets with calcium carbonate

Table 2. Characteristics of tablets with calcium carbonate or calcium citrate

Selected characteristic	Tablets with calcium		Requirement
	carbonate	citrate	
Average calcium content (mg ± SD)	100.0 ± 1.4	100.0 ± 2.7	consistent
Average pill weight (mg ± SD)	340.0 ± 2.3	800.0 ± 7.2	consistent
Friability (% ± SD)	0.8 ± 0.1	0.9 ± 0.1	< 2%
Hardness (N ± SD)	102.4 ± 10.8	132.8 ± 13.5	–
Disintegration time (min ± SD)	4.0 ± 0.2	13.0 ± 0.3	< 15 min

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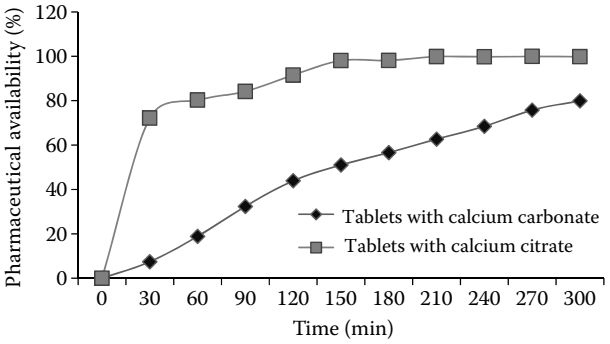

Figure 1. Calcium release profiles from tablets with calcium carbonate or calcium citrate ($P < 0.05$)

was significantly lower than the release from tablets with calcium citrate. After 5 h ~80% of calcium was released from tablets containing calcium carbonate and 99.9% from tablets containing calcium citrate. From tablets with calcium citrate ~79% of calcium was released after 30 min and ~100% after 3 hours. From tablets containing calcium carbonate ~7% calcium was released after 30 min and only ~60% after 3 hours. These results suggest that calcium in the form of calcium citrate has high pharmaceutical bioavailability which has an impact on its more efficient supplementation. Calcium from tablets containing calcium carbonate was released at the speed k = 0.32 h^{-1} and from tablets containing calcium citrate nearly 4 times faster (k = 1.38 h^{-1}). The half-time release for tablets containing calcium citrate was $t_{50\%}$ = 0.5 h and from tablets containing calcium carbonate $t_{50\%}$ = 2.2 hours. These results can be connected with different solubility of calcium salts. The solubility of non-organic calcium carbonate depends on pH of the environment. There are also some excipients in the composition of both preparations. It is inulin in tablets containing calcium carbonate which acts as a binder in tablets containing calcium carbonate and as a diluent in tablets containing calcium citrate. It is suggested that inulin added to the daily diet (8.0 g/day) can increase (15–20%) calcium and magnesium absorption in young people and women after menopause, as well as bone mineralisation (Dolińska *et al*. 2008b). According to the previous research where an *in vitro* model was used to simulate the intestinal permeation of calcium depending on the type of salt, its concentration and pH of acceptor, the permeation of ions from calcium carbonate was at the level of 9.6–100%, fumarate 18.3–81.2%, citrate 17.7–79.5%, and gluconate 21.2–81.0% (Dolińska *et al*. 2011a, b). The proper conditions of salt absorption and tablet composition can provide higher calcium availability and as a result higher supplementation efficiency (Dolińska *et al.* 2011a, 2012).

CONCLUSION

Shells of chicken eggs are an interesting alternative to the currently used products in supplementation of other natural sources of calcium to humans and animals. Higher solubility of calcium carbonate from the shells of chicken eggs, compared to carbonate derived from oyster shells, and the presence of valuable mineral components (strontium, barium) make them an excellent biomaterial for the production of new dietary supplements (Szeleszczuk *et al*. 2015). In addition, the conversion of calcium carbonate, calcium citrate results in a calcium salt with improved properties compared to calcium carbonate. Calcium citrate obtained from chicken eggshells is characterised by a suitable microbiological purity and includes valuable minerals in its composition (Dolińska *et al.* 2011a, c). The study of the kinetics of calcium release to the artificial gastric juice confirms that calcium is more rapidly released from the tablets containing calcium citrate derived from eggshells than from those with synthetic calcium carbonate.

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