

Calcium carbonate as modifier of mechanical properties of alginate/Ca microbeads

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INTRODUCTION

Over the last three decades the polyelectrolyte complexes, due to unique formation under physiological conditions and potential applications as microcapsules for bioimmobilization of different living cells, with a variety of different approaches and systems based on various polymer chemistries, processes and encapsulation technologies have been evaluated. Among them still the most popular is one-step process based on calcium/alginate polyelectrolyte complex formation. However, one major drawback of this system is fact that it is not thermodynamically stable, where with time the polyvalent cations within the complex are gradually replaced by monovalent ones leading first to continuous decrease of the capsule mechanical resistance and increase of their porosity, and finally dissolving (disintegration) of the all system. In most cases during the storage of microcapsules at pH higher than 6,0 in carbonate or phosphate based buffer solutions one can observe the ageing process. This is particularly critical during long term application in physiological solutions. Therefore, numerous groups all over the world have been looking for the best way to improve the mechanical stability of alginate/calcium beds. For example the modification of this chemistry has been extensively investigated, where metal cations other than Ca^{2+} and additional coating using natural or synthetic polyelectrolytes have been employed.

The aim of this study was to investigate the influence of calcium carbonate as potential active additive and reservoir of calcium cations, which could improve/extend the mechanical stability of alginate/Ca microbeads especially during storage in various pH/salt solution systems. Besides, we have decided also to investigate the rate of calcium ions exchange between Ca-alginate hydrogel microbeads and medium depending on selected conditions.

MATERIAL AND METHODS

Materials: The following materials have been applied for capsule preparation: Sodium Alginate – Keltone HVCR (ISP, Germany) food grade with molar mass (MM) of $M = 440,000 \text{ g/mol}$. Calcium Chloride hexahydrate pure p.a. (Chempur, Poland), was used as cross - linking agents/solution. Calcium Carbonate pure p.a. (Chempur, Poland) in powder form with average diameter of $50 \mu\text{m}$ (Mastersizer 2000, Malvern UK), was used as an additional source of calcium ions. Buffer acetate (pH 6,0); buffer phosphate (pH 6,0); 0,155M sodium chloride and potassium chloride solutions.

Capsule Formation: All microcapsules were prepared at room temperature using classical one-step procedure. A specific amount of calcium carbonate (1,5g) was mixed with a 100 ml of aqueous solution of sodium alginate (1,5% wt. %) and stirred using a magnetic agitator (Heidolph MR 3001) at 900 rpm for 30 min in order to form an uniform alginate-calcium carbonate suspension. Then 30

cm^3 alginate/ CaCO_3 suspension was dripped through the needle of diameters 0,8 mm with a syringe directly into 600 cm^3 aqueous 0,155 M solution of CaCl_2 . After 15 min of hardening, the formed alginate beads were separated from the calcium chloride solution and next they were washed two times for 10 min with distilled water. The prepared capsules were stored at room temperature in seven different solutions including acetate buffer (pH 6,0); phosphate buffer (pH 6,0; 6,5; 7,2 and 7,4); 0,155M sodium chloride solution 0,155M and potassium chloride solutions and demineralized water. As control in all experiments samples Ca-alginate beads without calcium carbonate addition were applied.

Mechanical Properties Characterization: The mechanical resistance determination were performed using standard compression test at 0.1 mm/s mobile probe speed until bursting was observed (Zwick/Roell Z 2.5, Germany). The force exerted by the probe on the capsule was recorded as a function of the compression time. Ten capsules per batch were analyzed in order to obtain statistically relevant data. The measurements were surveyed after 1, 3 and 7 days capsules storage.

Calcium ions measurement: The concentration of calcium ion were measured using a calcium-selective electrode - ISE (Eutech, Singapore). The measurements were performed for each system after 1, 2, 3, 4 and 7 days. After each measurement each solution was replaced with fresh one, respectively.

RESULTS AND DISCUSSION

The effect of calcium carbonate as additive on the properties of binary polyelectrolyte alginate/Ca beads can be observe both visually (Fig. 1) and instrumentally when one compare mechanical resistance of microbeads stored in demineralized water (Fig. 3). In case of microbeads prepared in presence of 1,5% calcium carbonate they are opaque and have at least 30% higher mechanical resistance in comparison to pure alginate/Ca system.

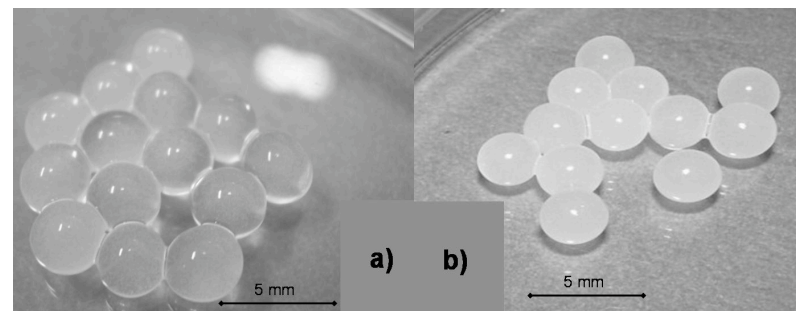


Fig.1. Photographs of two types of different microcapsules: a) without CaCO_3 in demineralized water; b) with 1,5% CaCO_3 stored in acetate buffer solution of pH 6,0.

Both systems after the formation have been stored in five different solutions (Fig. 3). For most of them already after 1 day of seasoning, one can observe a significant reduction of mechanical resistance. Due to the relatively “weak” type of the chemical bonds between charge carboxylic groups of alginate and calcium cations, resulting microcapsules are very sensitive to environmental conditions and, therefore tend to change their properties during storage in solution of monovalent

metal salts specially when pH was above 6,5 (Fig. 2). After 3 days only microbeads stored in water and system with calcium carbonate stored in acetate buffer of pH 6,0 were still mechanically stable.

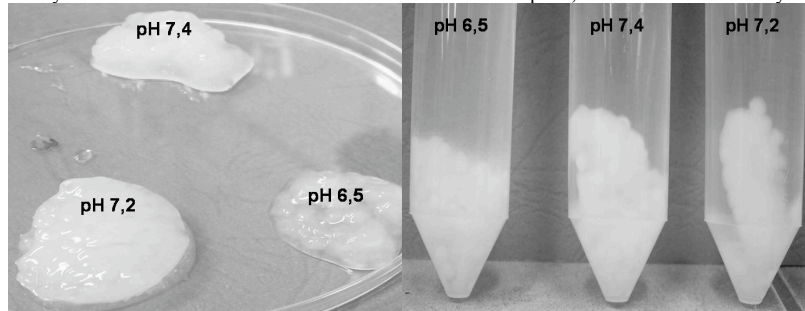


Fig. 2. Calcium/alginate microcapsules with 1,5% CaCO₃ stored in buffer phosphate solutions of various pH: 6,5; 7,2 and 7,4

All these three systems were stable even after 7 days of storage. In case of both types of microbeads kept in solution of pH above 6,5 there was no effect of addition of calcium carbonate, due to poor solubility of it in pure water and solution of pH above 7,0.

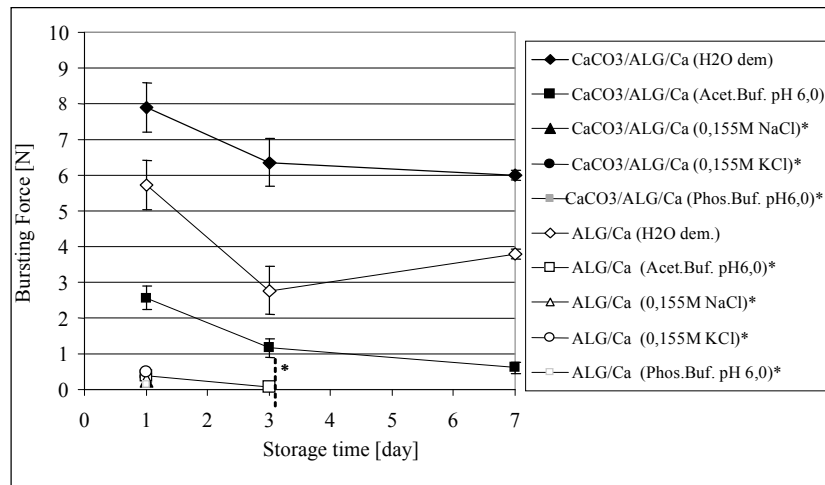


Fig. 3. Change of mechanical resistance of two types of microbeads during storage in different conditions (different pH and type of salt); *) indicates systems which have completely disintegrate after 3 days of storage).

In case of pH 6,0 solubility of calcium carbonate in pure water is high enough, so calcium ions could potentially participate in complex formation with alginate carboxyl groups, but in case when phosphate buffer is present the insoluble calcium phosphate will be formed. Although when one is applying instead phosphate and acetate buffer the resulting very soluble calcium acetate is formed, so calcium cations can participate in complex formation with carboxylic groups of alginate and as result more stable capsule are formed (Fig. 4). This potential mechanism of alginate/Ca complex

stabilization has been confirmed by measurements of free Ca²⁺ ion concentration, where only in case of aforementioned systems with calcium carbonate stored in acetate buffer one can observe the continuous increase of calcium ions in solution after second day of storage due to continuous solubilization of calcium carbonate.

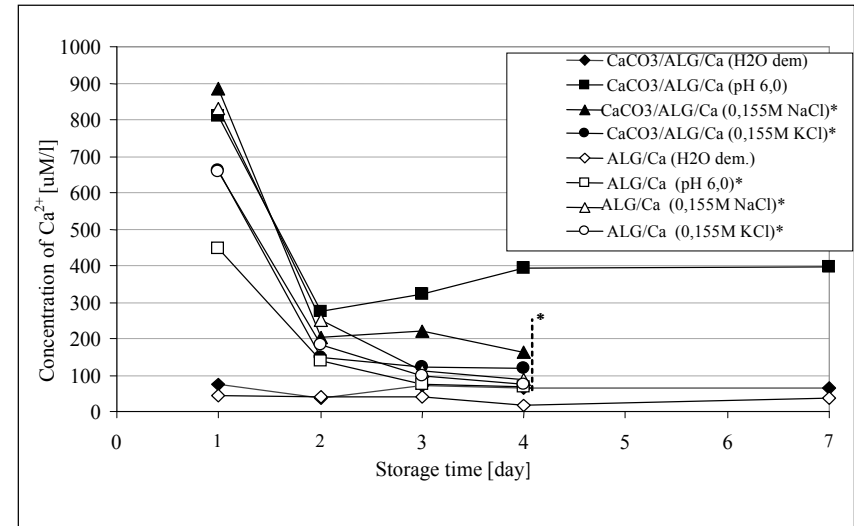


Fig. 4. Concentration of Ca²⁺ cations during storage of two types of microbeads in different conditions (different pH and type of salt); *) indicates microbeads which have completely disintegrate after 4 days of storage.

CONCLUSIONS

Calcium carbonate can be applied as potential stabilizer to improve mechanical resistance of calcium/alginate beads only during storage at specific pH/salt solution buffer systems.

One very important factor which influence calcium release profile and mechanical resistance of microcapsules is pH and type of dissolved salts in storage buffer solution.

This results clearly proved that not only monovalent metal cations like sodium and potassium, but also some anions like phosphate can leads to ion exchange and disintegration of calcium/alginate carboxylate complex.

As next step we are planning to investigate the effect of calcium carbonate concentration on mechanical properties and release kinetics of calcium ions.

Furthermore, the effect of calcium carbonate on porosity and cut-off oh hydrogel membranes as well their long term biocompatibility towards specific bacteria cells with optimum pH around 6,0 has to be investigated.