**Chitosan-alginate matrices as the carriers for the liposomal medical products**

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## INTRODUCTION

The article represented is devoted to research of ability of chitosan-alginate matrices as the carriers for the liposomal medical products application. It can be important for creation of delivery systems of proteins, vaccines, antibiotics etc. (Ramadas M. et al. (2000), Dai C. et al. (2005).

The procedure of obtaining of liposome-containing capsules with different composition of coats, stable in medias, simulated conditions of gastrointestinal tract was developed. Capsules are the liposome-containing capsules on the basis of calcium alginate or barium alginate, on which surface sequentially layer of calcium (barium) alginate applied. Thereby generated double-layer coat protects capsules of dissolving in acidic medium of stomach, whereas superficial calcium alginate or barium alginate layer is stable in medias with pH < 7.0. In medias with pH > 7.0 (intestine) this layer dissolving, exposing alginate-chitosan layer, that is steady enough in alkaline media and promotes prolongation of liposomes emission from capsules core. By varying chitosan molecular weight and its properties, it is possible to change the rate of capsules dissolving in lowest part of gastrointestinal tract in defined range, and, therefore, the rate of drug «loaded» liposome arrival in organism.

Similar capsules can be used at liposomal protein drugs for oral use creating,

## MATERIALS AND METHODS

Alginic acid Sodium salt from brown algae was obtained from Fluka (loss on drying ≤ 15 %; ash ≤ 30 %; pH (10 mg/ml $H_2O$) 6.0 – 8.0). Phosphatidylcholine liposomes («Nanophospholip») were obtained from Institute of biomedical chemistry (Moscow, Russia). All other reagents used were of analytical grade.

In this research chitosan of different viscosity was used: chitosan low-viscous (< 200 mPa·s, 1 % in acetic acid, $20^0$ C) and chitosan middle-viscous (200 - 400 mPa·s, 1 % in acetic acid, $20^0$ C). The samples were obtained from Fluka.

## RESULTS AND DISCUSSION

Phosphatidylcholine liposomes were dispersed in 2.0 % (w/v) solution of sodium alginate. The dispersion obtained was added drop by drop (by syringe with a needle) to 1.0 % (w/v) chitosan solution in 1.0 % acetic acid and held in this solution for 25 minutes. During this time interval the ionic interaction between polysaccharides takes place, resulting to formation of spherical capsules with a core containing viscose sodium alginate solution and chitosan-alginate hydrogel external coat.



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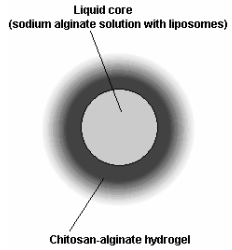
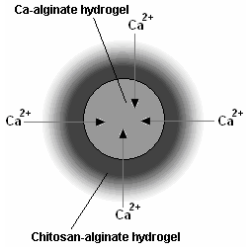
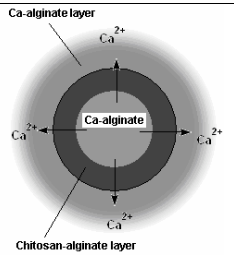
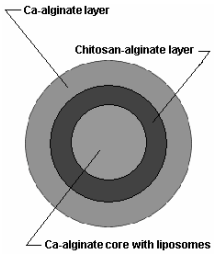
| Phase of process | Capsule structure | Process description |
|---|---|---|
| Holding in chitosan solution |   | While sodium alginate solution dropping in chitosan solution, the hydrogel layer forms on the drop surface due to ionic interaction. Large chitosan molecules can't penetrate through this layer into the capsule, therefore the capsule core corresponds the solution of unreacted sodium alginate. |
| Addition of calcium- or barium alginate powder to solution |   | Bivalent calcium- or barium cations diffuse in capsule center, where the interaction with sodium alginate occure, resulting to calcium- or barium alginate hydrogel formation. Simultaneously the saturation of all capsule volume by surplus calcium- or barium cations takes place. |
| Capsules holding in sodium alginate solution |   | Surplus calcium- or barium cations leave the capsule and react with surface alginate macromolecules, generating additional coat over chitosan-alginate layer. |
| Capsules holding in calcium- or barium chloride solution |   | In calcium chloride solution the solidification of external layer of calcium- or barium alginate hydrogel take place. As a result the two-layer coated capsule obtained. |

**Table 1**

In 25 minutes into capsule-containing chitosan solution calcium- or barium alginate powder was entered in amount required for obtaining of 3.0 % solution at total dissolution of reagent. After

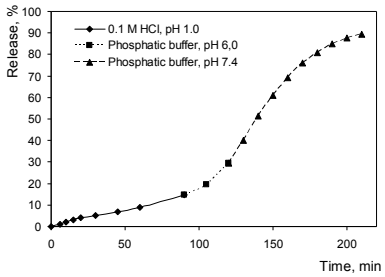



**Figure 1: Ca-alginate and chitosan low-viscous**

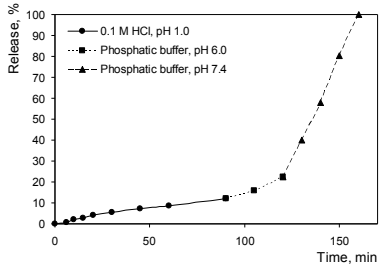



**Figure 2: Ca-alginate and chitosan middle-viscous**

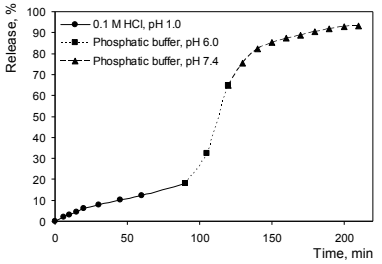



**Figure 3: Ba-alginate and chitosan low-viscous**

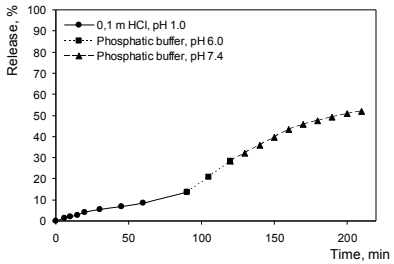



**Figure 4: Ba-alginate and chitosan middle-viscous**

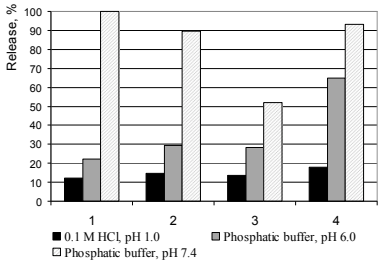



**Figure 5: 1 – Ca-alginate and chitosan middle-viscous; 2 - Ca-alginate and chitosan low-viscous; 3 - Ba-alginate and chitosan middle-viscous; 4 - Ba-alginate and chitosan low-viscous**

$CaCl_2$ or $BaCl_2$ dissolving the capsules were held in solution obtained during some more 25 minutes. At the same time, due to calcium or barium cation diffusion the formation of calcium- or barium alginate hydrogel in capsule core was occurred with simultaneous gel saturation by surplus amount $Ca^{2+}$ or $Ba^{2+}$ cations.

Then the capsules were extracted from the solution and carried into 1.0 % (w/v) sodium alginate solution, where they were incubated for 5 minutes. During this time interval the surplus calcium- or barium cations leaved the capsules and interacted with alginate macromolecules, forming the calcium- or barium alginate hydrogel layer on capsules





surface. To fix this layer the capsules were repeatedly incubated in 3.0 % calcium- or barium chloride solution for 10 minutes.
Then the capsules were extracted from the solution, washed three times by distilled water and dried in desiccator at 30 - $40^0$ C till retention constant weight. Dried capsules were kept in airproof glass or plastic boxes.

The phases of capsules obtaining process are shown schematically in table 1.

Capsules with phosphatidylcholine liposomes were placed in mediums with the temperature of $37^0$ C. As mediums used sequentially:

- 0.1 M hydrochloric acid (pH = 1.0, 90 min);
- phosphatic buffer (pH = 6.0, 30 min);
- phosphatic buffer (pH = 7.4, for complete solution of capsules).

It was shown that not only the nature of chitosan used, but the metal cation type also effects the release of phosphatidylcholine liposomes. The shapes of phosphatidylcholine liposomes release from capsules with different coats are represented on fig. 1 - 4. General rate of phosphatidylcholine liposomes release in different medias is shown on fig.5. The capsules with coats based on calcium- or barium alginate combined with chitosan middle-viscous are characterized by lowest rate of release in acidic media. Ba-alginate combined with chitosan middle-viscous is characterized by low rate of release of liposomes in all medias including medias pH > 7.0. At the same time, calcium alginate combined with chitosan middle-viscous in such medias is able to dissolve rapidly releasing encapsulated liposomes (fig. 2). The rest combinations possess different ability to liposomes releasing in various medias.

## CONCLUSIONS

Thereby, using combination of calcium- or barium alginate hydrogel with various viscosity chitosan it is possible to control the releasing of liposomes drug «loaded» in intestine or stomach conditions. It allows to decrease drug dose and to increase the therapeutic influence duration. Moreover, peptide drugs encapsulation in alginate and chitosan based matrixes provides the protection from ferment destruction.

## REFERENCES


- Ramadas M. et al. (2000) *Lipoinsulin encapsulated alginate-chitosan capsules: intestinal delivery in diabetic rats.* J. Microencapsulation 17 (4) 405 – 411.

- Dai C. et al. (2005) *Factors affecting protein release from microcapsule prepared by liposome in alginate.* Colloids and Surfaces B: Biointerfaces 42 253 – 258.