Bulgarian Chemical Communications, Volume 56, Special Issue D1 (pp. 158-162) 2024 DOI:10.34049/bcc.56.D.S1P72

Effect of different hydrocolloids on Ca (II) – alginate beads containing extracts from *Arthrospira platensis*

N. T. Petkova[1], Kr. T. Nikolova[2*], G. D. Gentscheva[3,4], A. Ts. Gerasimova[5], D. G. Kovacheva[4], N. K. Panova[2], St. At. Vladimirova[6]

[1]*Department of Organic Chemistry and Inorganic Chemistry, University of Food Technologies, Technological Faculty, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria*
[2]*Department of Physics and Biophysics, Medical University of Varna, Faculty of pharmacy, 84 Tzar Osvoboditel Blvd., 9002 Varna, Bulgaria*
[3]*Department of Chemistry and Biochemistry, Medical University - Pleven, Faculty of Pharmacy, 1 Saint Kliment Ohridski Str., 5800 Pleven, Bulgaria*
[4]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Bl. 11, 1113 Sofia, Bulgaria*
[5]*Department of Chemistry, Medical University of Varna, Faculty of Pharmacy, 84 Tzar Osvoboditel Blvd., 9002 Varna, Bulgaria*
[6]*Institute of Physical Chemistry, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Bl. 11, 1113 Sofia, Bulgaria*

Received: November 3, 2023; Revised: April 11, 2024

Alginate was applied in food matrix, and pharmaceutical products for efficient encapsulation of bioactive compounds or as a drug carrier. In the current study phycocyanin isolated from dry *Arthrospira platensis/Spirulina* was encapsulated in alginate beads. The aim of the current research was to evaluate the effect of different hydrocolloids (inulin, pectin and guar) at different pH values and temperatures on phycocyanin content in the alginate beads. The alginate microspheres were formed with 1% sodium alginate, 6% sucrose, 1% phycocyanin, and selected hydrocolloids, as neutral polysaccharides (inulin and guar) and anionic heteropolysaccharides (pectin). Moreover, the diameter of alginate-phycocyanin beads with or without hydrocolloids was evaluated. The addition of sucrose and inulin in a concentration of 6 % to alginate-phycocyanin beads stored at 25 and 37 °C showed the highest content of phycocyanin. The phycocyanin loss was minor at pH 6 and pH 9, respectively, especially when 0.8% pectin or 6% inulin was added to the alginate matrix. XRD patterns of all beads showed amorphous humps situated at different angles, depending on the particulate type of the hydrocolloid. The current research evaluates the potential of alginate beads with phycocyanin, as well as with pectin, guar and inulin to be used in food and pharmaceutical products.

Keywords: Ca (II) - alginate beads, phycocyanin, hydrocolloids, supplements

INTRODUCTION

Alginate is often used to create biodegradable microspheres that can be used to encapsulate unstable substances used in pharmaceuticals and food technology. In this way, unstable substances are protected from the pH of the environment in which they are used and/or from the action of enzymes contained in them [1]. As an anionic polysaccharide consisting of various ratios of L-guluronic and D-mannuronic acids linked by glycosidic bonds, alginate also has some disadvantages [2]:

- low encapsulation efficiency of the transported substance;
- rapid release of charged molecules.

The mentioned disadvantages are caused by the high porosity of the gel, which leads to leakage of the included medicinal products or phytopreparations from the alginate beads. The development of alginate microspheres with different hydrocolloids improving their properties, becomes more and more urgent task with the increasingly frequent use of novel drug delivery systems in pharmaceutical technologies. Microspheres - carriers of phytopreparations or medicinal substances, are essential for the transport and release of the substance at the target site with a controlled rate and good bioavailability [3]. Moreover, the addition of different hydrocolloids increases the stability of alginate gel and enhances the encapsulation process [4, 5]. Incorporation of natural polymers to alginate beads such as pectin [6], chitosan [7], inulin, cellulose, gelatin [8, 9] is an advantage not only due to their abundance in nature and the associated low price of the product, but also to the fact that they are low-toxic, biodegradable and biocompatible [10, 11]. The current research is aimed to evaluate the

* To whom all correspondence should be sent:
E-mail: kr.nikolova@abv.bg

effect of different hydrocolloids (inulin, pectin, and guar) at different pH values and temperatures on phycocyanin content in alginate beads.

MATERIALS AND METHODS

Materials

Sodium alginate with M-block 61%, G-block 39 %, and M/G ratio of 1.55 (Sigma-Aldrich, Munich, Germany), $CaCl_2$ and sucrose (Sigma-Aldrich, Munich, Germany). Inulin (Raftiline®HPX Beneo, Orafti, Belgium) with degree of polymerization DP = 25. Citrus pectin with a medium degree of esterification (DE = 70.6 %) originating from Spain. Guar gum was supplied from Orion (Bulgaria).

Phycocyanin extract

Dry *Arthrospira platensis* was purchased from the bioreactor (Varvara, Bulgaria). Phycocyanin was extracted in ultrasonic bath (IsoLab, Wertheim, Germany) at frequency 40 kHz, temperature 40 °C for 1 h [12]. The obtained water extract was filtered and lyophilized.

Preparation of Ca(II)-alginate beads with phycocyanin and different hydrocolloids

The calcium alginate beads encapsulated with phycocyanin and different hydrocolloids were prepared by the method of Petrova et al. [13] with slight modification. The resulting suspension of 1 % sodium alginate, 6% sucrose and 1 % phycocyanin was heated at 40 ºC for 3 min and homogenized with a laboratory Homogenizer IKA T18 (IKA – Werke GmbH & Co. KG, Staufen, Germany) for 2 min. Four different samples with 6% sucrose were prepared as follows:

A) control sample 1% alginate + 1% phycocyanin;

B) 1% alginate + 1% phycocyanin + 6.0 % inulin;

C) 1% alginate + 1% phycocyanin + 0.8% pectin;

D) 1% alginate + 1% phycocyanin + 0.6% guar.

To remove air bubbles, the obtained suspension was placed in the fridge for 60 min. The alginate beads were prepared as liquid solutions and were transferred into a syringe and dropped into a cold 2 % water solution of $CaCl_2$ at a temperature of 7 ºC. The obtained Ca(II)-alginate beads with phycocyanin and different hydrocolloids were filtered, washed twice with distilled water, and placed in containers with distilled water for further use.

Release of phycocyanin

24 hours after preparation, the absorption spectrum of the water with which the beads were flooded, was measured in order to determine the most suitable formulation for retaining phycocyanin.

Equal amounts of microspheres were placed in two different media at two different temperatures 25 ºC and 37 ºC - 25 mL acetate buffer with pH 6.0 and 25 mL ammonium buffer solution pH 9.0. The working solutions are obtained by mixing 25 pieces of the corresponding beads with 25 mL of a buffer solution with the corresponding pH. One series of solutions is left at 25 °C, and the other series of solutions is tempered for 30 min at 37 °C. The samples were taken 30 min after the establishment of thermal equilibrium maintained by a water bath.

The absorption spectra of the samples were measured in the visible range using a UV-VIS spectrophotometer. The phycocyanin content in the solution was determined according to the formula [14]:

$$phycocyanin(mg\ mL^{-1}) = \frac{A_{615nm} - 0.474 \times A_{652nm}}{5.34} \quad (1)$$

Average particle size

The average diameter and shape of the alginate microspheres were determined with a stereomicroscope SZ61 TR (Evident Corporation, Nagano, Japan). The spheres were observed in a bright field with reflected light at a 10 × objective and 4.5 × eyepiece magnification. Photos were taken with a 5Mp Wi-Fi camera Olympus EP50. The dimensions of the alginate spheres were determined using EPview software.

Morphology

For SEM and X-ray analyses the spheres were left to dry in open air at room temperature for 2 weeks.

The morphology of the dried alginate microspheres was examined using scanning electron microscopy (SEM) on a scanning electron microscope JEOL JSM-6390 (Tokyo, Japan). Samples were mounted on a metal holder using a sticky carbon band with increased conductivity and coated with gold. Accelerating voltage 20 kV, magnification ×10000 in secondary electrons.

X-ray analysis

Patterns of the alginate microspheres were collected within the range from 5.3 to 80° 2θ with a constant step 0.02° 2θ and counting time 52.5 sec./step on Bruker D8 Advance diffractometer (Germany) with Cu K_α radiation and LynxEye detector. Phase identification was performed with the Diffracplus EVA using ICDD-PDF2 (2014) database.

RESULTS AND DISCUSSION

The dimensions of 20 alginate spheres of each type were determined using an optical microscope. Table 1 presents the mean diameter ± standard deviation.

Table 1. Characterization of the size of alginate beads with phycocyanin.

Formulation	Polysaccharide concentration, %	Length ± SD	Width ± SD
		µm	µm
Sample A	Alginate 1	3791 ± 236	3661 ± 101
Sample B	Alginate 1 - Inulin 6.0 %	3466 ± 203	1978 ± 116
Sample C	Alginate 1 - Pectin 0.8 %	4486 ± 624	4485 ± 626
Sample D	Alginate 1 - Guar 0.6 %	3749 ± 103	3726 ± 103

The shape of the beads is shown in Figure 1.

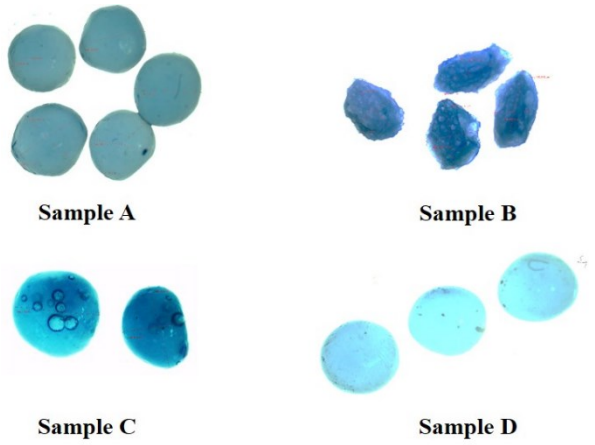


Figure 1. Shape of the alginate beads imaged with an optical reflection microscope.

The control samples with alginate and phycocyanin, as well as with alginate, phycocyanin, and guar (sample D), have an almost spherical shape, and a smooth surface with slight "wrinkles" on it. The samples with phycocyanin, alginate and pectin (sample C), similar to the above-mentioned samples, have a smooth surface and a spherical shape, but air bubbles are clearly observed under the surface layer of the beads. This can be related to the increasing viscosity of the sample when pectin is added. The alginate-phycocyanin-inulin samples (sample B) were irregularly, star-shaped, and were much smaller than the other beads.

The relatively large size and spherical shape is determined by the low concentration of calcium dichloride (2%) in the preparation of the alginate spheres. A low concentration of calcium leads to a higher water content in the gel and a limitation of gelation [15]. As the concentration of calcium dichloride increases, the size of the spheres decreases due to an increase in cross-linking [16].

The X-ray spectra of the investigated samples are presented in Figure 2.

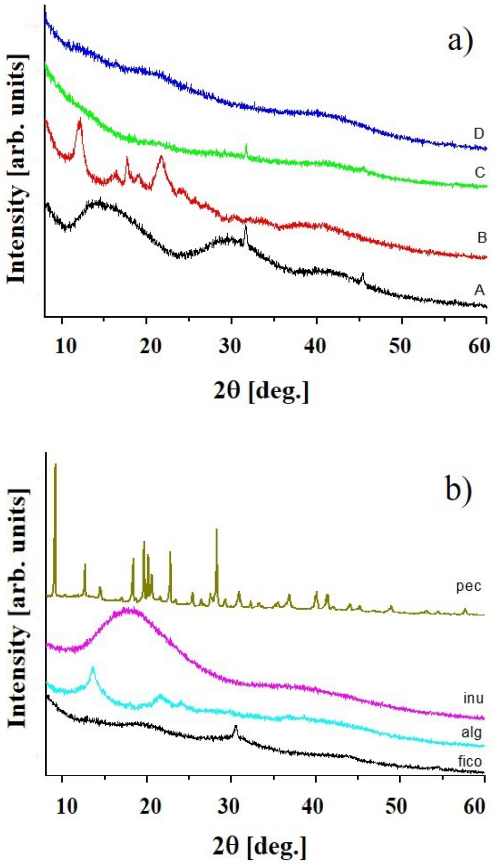


Figure 2. X-ray spectra of b) starting substances and a) alginate spheres with additives respectively: A) control sample 1 % alginate + 1 % phycocyanin; B) 1 % alginate + 1 % phycocyanin + 6.0 % inulin; C) 1 % alginate + 1 % phycocyanin + 0.8 % pectin; D) 1 % alginate + 1 % phycocyanin + 0.6 % guar.

In the X-ray pattern of citrus pectin, peaks of highly crystalline residues of glucose (ICDD-PDF#00-24-1964) and glucose-hydrate (ICDD-PDF#00-30-1736) are observed, while inulin shows a typical amorphous pattern consisting of one “hump” with a maximum at around 18.5 º 2θ.

Na-alginate is characterized by a semi-crystalline diffraction pattern with the strongest peaks at 13.4º, 21.5º and 24.1º 2θ, which correspond to G(110), G(200)+M(020) and M(200) reflections from G and the M-blocks of the alginate structure [17, 18].

The pattern of phycocyanin comprises an amorphous component and a very small amount of a crystalline phase identified as (Na, K) Cl (ICDD-PDF#01-075-0303).

The X-ray pattern of the alginate spheres with phycocyanin (A) shows amorphous peaks, which

indicates that interaction of the two components has occurred. A small amount of crystalline phase identified as NaCl was also detected (ICDD-PDF#01-080-3939).

The X-ray pattern of the sample with alginate, phycocyanin, and inulin (Sample B) presents a semi-crystalline sample in which the alginate peaks are preserved, perhaps indicating the stronger interaction of phycocyanin with inulin.

In the sample of phycocyanin, alginate, and pectin (C), as well as in that of phycocyanin, alginate, and guar (D), amorphous diffraction patterns are observed. A small amount of crystalline NaCl (ICDD-PDF#01-080-3939) was also detected in the phycocyanin, alginate, and pectin samples.

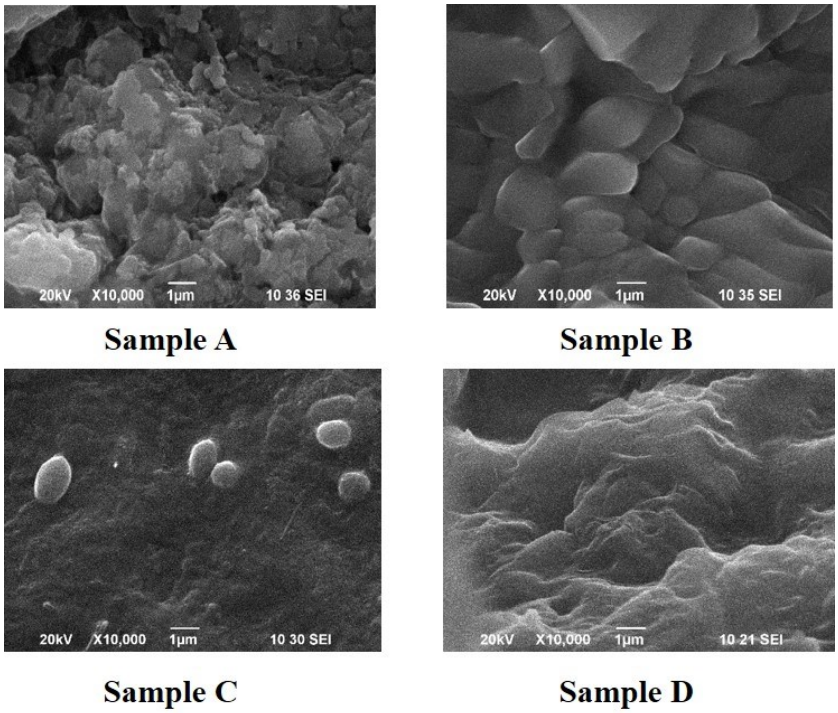
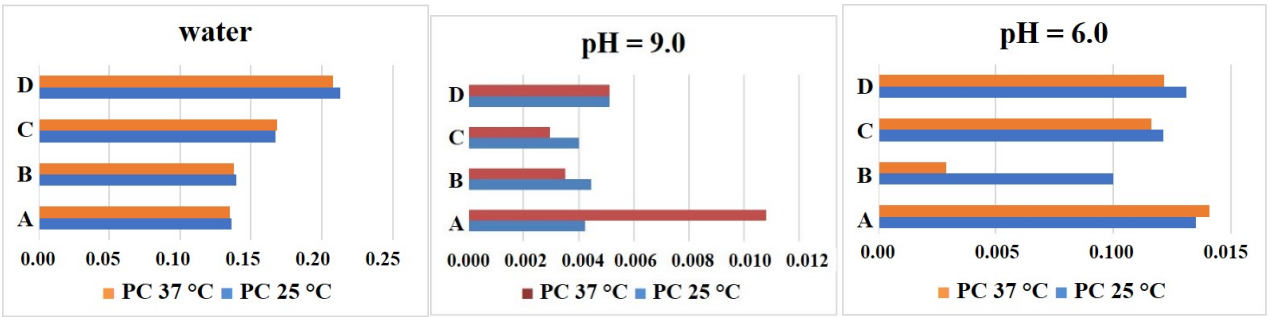


Figure 3. Morphological structure of the surface of the alginate beads.



A B C

Figure 4. Release of phycocyanin from alginate microspheres.

More detailed information on the morphological structure of the surface of the spheres was obtained from scanning electron microscopy (SEM) of the dried samples. The results are presented in Figure 3. After drying at room temperature, the microspheres lose their spherical shape, and their surface looks different depending on the constituents of the spheres. Sample A is characterized by a rough surface morphology, which is made up of irregularly shaped particles aggregated into larger structural motifs with gaps (pores) between them. In sample B, the particles are larger with a slightly elliptical shape with smooth edges and adhere to each other. The surfaces of samples C and D are smoothed, without clearly defined particles, and in sample C, spherical particles are also observed above the surface, probably as a result of the transformation of air bubbles during the drying of the sample.

The results of phycocyanin release are shown in Figure 4.

The release of phycocyanin in an aqueous environment after a 24-hour stay of the alginate pearls was investigated to study the possibility of using them in smoothies or juices as a functional additive. In aqueous media, the release of phycocyanin at room and body temperature was almost the same for the control sample and that with added inulin, followed by that with pectin. The retention of the substance by alginate spheres with guar is the weakest.

With the ammonium buffer, except for the control, for all other samples the release of phycocyanin after 30 minutes at a temperature of 37 °C was weaker than at a room temperature of 25 °C.

In the case of the acetate buffer, the weakest phycocyanin loss was observed in the samples with inulin at 37 °C. Therefore, inulin incorporated in alginate beads fixed well phycocyanin into alginate-inulin matrices.

CONCLUSION

From the obtained results can be seen that inulin addition to alginate gel stabilized phycocyanin in the matrix and preserved it at all pH and water solutions. Therefore, this polysaccharide is proper as an encapsulating agent for phycocyanin together with alginate. In water solutions, the concentration of phycocyanin can reach 0.2 mg/ml when guar is used together with alginate. The most efficient encapsulating agent is 1 % alginate and 1 % alginate with 6 % inulin (Sample B). At pH 9 most efficient are pectin and inulin added to 1 % alginate (Samples B and C), while at pH 6 the phycocyanin is retained better in 1 % alginate and 6 % inulin (Sample B).

Acknowledgement: *This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0009-C02.*

REFERENCES

1. S. Onal, F. Zihnioglu, *Artif. Cells Blood Substit. Imm. Biotech.,* **30 (3),** 229 (2002).
2. A. Halder, S. Maiti, B. Sa, *International J. of Pharmaceutics,* **302 (1–2),** 84 (2005).
3. S. Gujral, S. Khatri, *IJAPBC,* **2,** 2277 (2013).
4. R. Bhagwat, I. Vaidhya, *Int. J. Pharm. Sci. Res.,* **4,** 970 (2013).
5. I. Petrova, N. Petkova, A. Slavchev, T. Petrova, *Mater. Sci. Eng.,* 1031 (2021).
6. V. Pillay, M. P. Danckwerts, Z. Muhiddino, R. Fassihi, *Drug Development and Industrial Pharmacy,* **31**, 91 (2005).
7. Y. Murata, K. Tsumoto, K. Kofuji, S. Kawashima, *Chem. Pharm. Bull.,* **51**, 218 (2003).
8. P. F. Almeida, A. J. Almeida, *J. Control. Rel.,* **97**, 431 (2004).
9. M. S. Hamid Akash, K. Rehman, S. *Chen. Polym. Rev.,* **55**, 371 (2015).
10. K. M. Z. Hossain, U. Patel, I. Ahmed, *Prog. Biomater.*, **4**, 1 (2015).
11. P. Singh, D. Prakash, B. Ramesh, N. Singh, T. T. Mani, *Ind. J. Novel Drug Delivery*, **3**, 70 (2011).
12. I. Minchev, N. Petkova, I. Milkova-Tomova, *Biointerface Research in Applied Chemistry*, **11(2),** 9296 (2021).
13. I. Petrova, N. Petkova, A. Slavchev, T. Petrova, *IOP Conf. Series: Materials Science and Engineering*, 1031 (2021).
14. A. Bennett, L. Bogorad, *J. Cell Biol.,* **58**, 419 (1973).
15. J. R. Rodrigues, R. Lagoa, *J. of Carbohydrate Chem.*, **25**, 219 (2006).
16. H. Daemi, M. Barikani, *Scientia Iranica*, **19**, 2023 (2012).
17. S. Bhagyaraj, I. Krupa, *Molecules*, **25(3)**, 435 (2020).
18. J. Fabia, C. Z. Slusarczyk, A. Gawlowski, *Fibres. Text. East Eur.,* **13**, 114 (2005).