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Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
*J. Serb. Chem. Soc.***00(0)** 1-14 (2025)
*JSCS–13094*
*Original scientific paper*
Published DD MM, 2025



# Optimizing alginate immobilization of food-derived C-phycocyanin: structural and functional characterization

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(Received 25 October 2024; revised 7 January 2025; accepted 27 January 2025)

*Abstract:* C-phycocyanin (C-PC) represents a significant component of the cyanobacteria *Arthrospira platensis* (*Spirulina)* biomass. Beyond its nutritional value, this protein exhibits numerous beneficial biological activities. A covalently attached chromophore, phycocyanobilin, gives C-PC a blue color, enabling its use as a natural food colorant. Additionally, phycocyanobilin exhibits various bioactive properties, including metal-binding activities. A key drawback to the broader industrial application of C-PC is its poor stability. Alternative food formulations using natural polymers as carriers and active components have recently gained considerable scientific attention. This paper describes optimized conditions for C-PC immobilization using alginate. The structural stabilization of immobilized C-PC was analyzed under high temperature (60°C) and high pressure (450 MPa). The storage stability of immobilized C-PC in dried alginate beads was tested by keeping the samples at 4°C for one month. The potential application of immobilized C-PC for the removal of mercury ions was also investigated. Alginate immobilization proved effective in stabilizing C-PC, significantly preserving its structure during prolonged storage, thermal treatment, and high-pressure exposure. Under the tested conditions, 97% of $Hg^{2+}$ ions were removed by immobilized C-PC. Overall, this study optimized the procedure for enhancing C-PC stability through alginate immobilization and broadened its potential applications in food and bioremediation industries.

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https://doi.org/10.2298/JSC241025012G

*Keywords:* spirulina; C-phycocyanin; alginate; immobilization; stability; mercury ions.

## INTRODUCTION

Cyanobacteria *Arthrospira platensis*, commonly known as *Spirulina*, is a popular alternative food source considered a "superfood". The annual production of *Spirulina* reaches about 50,000 tons, with the majority produced in China.[1] *Spirulina* biomass is rich in proteins (over 50 %), lipids, vitamins, and minerals.[2] The major protein of *Spirulina* is the intensively blue-colored C-phycocyanin (C-PC).[3] This protein is part of multi-protein complexes known as phycobilisomes, which play a role in light-harvesting during photosynthesis.[4]

In its native state, C-PC is a hexamer with a $\alpha_6\beta_6$ structure. The building block of this hexamer is the αβ monomer, consisting of two subunits with molecular masses of 17.6 kDa for the α subunit and 18.1 kDa for the β subunit.[5] During assembly, three monomers form a trimer, which then combines with another trimer to form a hexamer in a face-to-face orientation.[6] The isolation of this protein yields a mixture of monomers, trimers, and hexamers, depending on the applied conditions and C-PC concentration.[7] The blue color of C-PC originates from the covalently attached tetrapyrrole chromophore phycocyanobilin (PCB). Each monomer of C-PC contains three PCB molecules, - one in the α subunit and two in the β subunit - attached *via* thioether bond to cysteine (Cys) residues.[8] Besides its direct role in light harvesting,[9] numerous beneficial biological activities of C-PC are associated with PCB, including antioxidant, anticancer, and anti-inflammatory properties.[10] Additionally, due to the presence of PCB, C-PC is capable of binding heavy metals, exhibiting dissociation constants in the nM range for mercury ($Hg^{2+}$) ions.[11]

The potential for industrial utilization of C-PC is significant. In addition to its nutritional value, its blue color makes it an attractive natural food colorant, especially as consumers increasingly seek natural ingredients in their diets.[12] Blue colorants are rare in nature, and synthetic alternatives are primarily used.[13] Both the Food and Drug Administration (FDA) and the European Union accept the usage of C-PC as a food colorant. A limiting factor for the broader utilization of C-PC lies in its poor stability.[14] C-PC from *Spirulina* is most stable in a pH range of 5.5 to 6. Its half-life rapidly decreases at temperatures between 47 and 64°C.[15] Numerous approaches for C-PC stabilization have been reported so far in the literature and are covered in several review papers. These include the addition of stabilizers in the form of small molecules like sugars, the presence of proteins, and encapsulation methods.[16] Encapsulation methods not only stabilize C-PC, but they are also potential approaches for creating novel, attractive types of food consisting of hydrogels packed with higher concentrations of nutritive proteins. Due to its vivid color, C-PC is particularly interesting in this regard.[17] Controlled release of proteins from encapsulating material is also an important benefit.

Alginate immobilization of C-PC has been reported to be cheap, efficient, and easy to fabricate and manipulate, and it also enables controlled pH release. While several papers on C-PC immobilization using alginate exist, they either involve combinations with other components besides alginate[18–20] or lack details about immobilization optimization regarding pH, protein structure after immobilization, and physical and chemical treatments, as well as the potential for prolonged storage without prior lyophilization of the alginate beads.[20–24] This paper aims to fill these gaps by fabricating alginate beads containing a higher concentration of C-PC and structurally characterized by absorption spectrometry after thermal and high-pressure treatments and prolonged storage at 4°C. Their potential application in bioremediation is also explored by testing their ability to bind mercury ions.

## EXPERIMENTAL

### *Materials*

All reagents were of analytical quality and were purchased from Sigma (Darmstadt, Germany). Commercial *Spirulina* powder was obtained from Nutrex (Hawaii, USA). C-PC was partially purified using the same procedure described in our previous paper.[25] Absorbance spectra of partially purified C-PC were recorded using LLG-uniSPEC 4 spectrophotometer (LLG labware, Meckenheim, Germany). Based on the absorbance spectra, the concentration of C-PC was determined to be 8.36 mg/mL using a published equation.[26] Its purity was measured to be 1.04 by the absorbance ratio at 620 and 280 nm,[27] indicating food-grade purity. Food-grade sodium alginate was purchased from Health Leads (Llandysul, United Kingdom).

### *Alginate immobilization of C-PC*

Alginate immobilization of C-PC followed similar published procedures,[20,22] with slight modifications. The final concentration of C-PC used for immobilization was 4.18 mg/mL. The alginate concentrations tested were 0.5, 1, and 2%, while the concentration of $CaCl_2$ solution was 2.5 %. $CaCl_2$ solution was prepared in distilled water. Equal volumes of alginate and protein solutions were mixed and then added dropwise to the $CaCl_2$ solution using a syringe with an 18G needle. The resulting beads were kept in $CaCl_2$ solution for 5 hours, then washed with the same $CaCl_2$ solution on a filter paper until no further blue color leached from the beads. The beads were stored at 4°C overnight in $CaCl_2$ solutions of different pH values: pH 1.35 (45 mM HCl), pH 2.75 (1.78 mM HCl), pH 4 and 5.5 (50 mM acetate buffer), and pH 7 (50 mM HEPES buffer). Beads were photographed the next day, and those with minimal C-PC leakage and no observable color change were selected for further analysis. For subsequent experiments, unless otherwise stated, beads were prepared with 1% alginate, 4.18 mg/mL of C-PC, and 2.5% of $CaCl_2$ in 50 mM acetate buffer, pH 4, except for $Hg^{2+}$ binding experiments, where 0.5% alginate was used. The fabricated beads were stored in solution at 4°C for no longer than 7 days until all experiments were conducted.

### *Temperature stability*

For the temperature stability test, alginate beads were placed in four separate microcentrifuge tubes with 1 ml of 50 mM acetate buffer, pH 4. Tubes were placed in a thermoshaker and incubated at 60 °C under 200 rpm for 30 min. Every 10 min, one microcentrifuge tube was removed. The control tube was incubated for 30 min at room temperature. After incubation, beads were placed in 1 ml 50 mM phosphate buffer, pH 7, and incubated at room temperature for 3 h, enabling swelling of alginate beads and C-PC leakage

into the solution, with concomitant recording of absorbance spectra of obtained solutions from 300 to 750 nm.

*High-pressure stability*

Alginate beads were placed in a plastic tube filled with 50 mM acetate buffer, pH 4, and sealed with parafilm to separate the sample from the water used as the pressure-transmitting liquid. The sample was placed in a custom-built stainless steel high-pressure cell at the Laboratoire Léon-Brillouin (Saclay, France) and connected to a pressure generator. The pressure was increased to 4,500 bar at 200 bar/min. After reaching the desired pressure, the sample was incubated for 30 min and then returned to atmospheric pressure. The beads were transferred to 10 mL of 50 mM phosphate buffer, pH 7, and incubated at room temperature for 3 h. Absorbance spectra were obtained as described in the temperature stability section.

*Storage stability*

Alginate beads containing C-PC were removed from the 50 mM acetate buffer and placed in a glass beaker without any solution. The beaker was covered with stretch film and stored in a fridge at 4°C for one month. The beads were then swollen by adding 20 mL of 50 mM phosphate buffer, pH 7, and after 3 h, absorbance spectra were recorded as in the temperature stability section. A control sample was made by placing a portion of alginate beads containing immobilized C-PC immediately into the same phosphate buffer for 3 h and recording the absorbance spectra of leaked C-PC as described in the temperature stability section.

*Removal of $Hg^{2+}$ ions by alginate beads containing C-PC*

The ability of C-PC in solution to bind $Hg^{2+}$ at pH 4 was first tested by determining the affinity constant using a FluoroMax spectrofluorimeter (HORIBA Scientific, Japan). Increasing concentrations of $HgCl_2$, from 50 to 700 nM, were added to 2.45 nM C-PC, and the emission spectra of C-PC were recorded from 600 to 750 nm, with excitation at 590 nm (PCB chromophore excitation). The buffer (50 mM acetate) spectrum was subtracted from the sample spectra. In this setup, no inner filter effect was observed, and the affinity constant was calculated using the following equation:

$$\log (F_0 - F / F) = n \log [L] + n \log K_a \quad (1)$$

where $F_0$ is the fluorescence of C-PC without $Hg^{2+}$, $F$ is the fluorescence of C-PC in the presence of C-PC, $[L]$ is the concentration of $Hg^{2+}$ in mol/dm$^3$, $n$ is the Hill coefficient, and $K_a$ is the affinity constant in $M^{-1}$.

The potential of mercury removal by C-PC immobilized into alginate beads was tested by adding 40 alginate beads to 15 mL of 1.75 ppm $HgCl_2$ solution in ultrapure water at pH 4. Control experiments were performed with the same number of alginate beads without C-PC, and an additional control solution of $HgCl_2$ was incubated without beads. The incubation lasted 48 h at room temperature under constant shaking at 200 rpm. $Hg^{2+}$ concentration in the samples was determined using an ICP-OES iCap 6500 Duo (Thermo Scientific, Waltham, USA).

*Statistical analysis*

All experimental results represent the averages of triplicates, with standard deviations not exceeding 5%. Statistical significance for $Hg^{2+}$ binding was confirmed using one-way ANOVA with Tukey's multiple comparison test, with $p$-value < 0.05 being considered statistically significant. The test was performed by using OriginLab software (Northampton, Massachusetts, USA), version 8.5.1. Absorbance spectra were first normalized to their maximum absorption values and then to the baseline.

## RESULTS AND DISCUSSION

### *Optimization of C-PC immobilization into alginate beads*

Using a straightforward procedure, alginate beads containing C-PC were successfully created. The beads exhibited an intense blue color due to the presence of native C-PC and maintained a spherical shape (Fig. 1).

Fig 1. Alginate beads with encapsulated C-PC.

By washing off unbound C-PC and calculating the concentration of both the unbound C-PC and that released in the $CaCl_2$ solution, it was estimated that more than 50% of the initial C-PC was successfully immobilized into alginate beads. This level of efficiency aligns with previously published results, indicating that alginate entrapment is an effective method for immobilizing substantial amounts of C-PC.[21,23,24] Interestingly, to the best of our knowledge, no published work has provided a detailed analysis of the pH conditions necessary for fabricating stable alginate/C-PC beads in solution.

When alginate/C-PC beads were incubated overnight at five different pH values, significant C-PC leakage was observed at pH 5.5 and 7 (Fig. 2, D–E), regardless of the alginate concentration. In contrast, no leakage occurred at acidic pH values of 1.35 and 2.75. However, the beads appeared green (Fig. 2, A-B), suggesting that the immobilized C-PC underwent structural alterations and protonation of the pyrrole ring in the PCB chromophore. At pH 4, the beads retained their intense blue color, with no detectable C-PC leakage under any of the tested alginate concentrations (Fig. 2C).

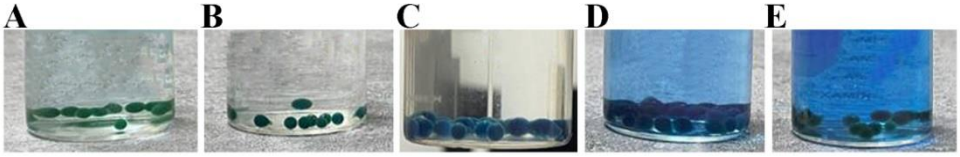


Fig 2. Alginate beads containing encapsulated C-PC incubated for 24 h at 4°C in 2.5% $CaCl_2$ solutions at pH 1.35 (**A**), 2.75 (**B**), 4 (**C**), 5.5 (**D**), and 7 (**E**).

It can be concluded that at pH 4, no significant structural alteration of the immobilized C-PC within the alginate beads, if any, occurred at all. Additionally, when these beads were transferred to a 50 mM phosphate buffer, pH 7, C-PC began to leak, turning the solution an intense blue, with no precipitation observed. The native state of C-PC was further confirmed through absorbance spectra (see Figure 4, for example). Interestingly, when alginate/C-PC beads incubated in solutions of pH 1.35 and 2.75 were placed in a neutral buffer, the blue color appeared in a solution containing beads made in pH 2.75, suggesting that structural alterations of immobilized C-PC under these conditions are partially reversible (Fig. 3).

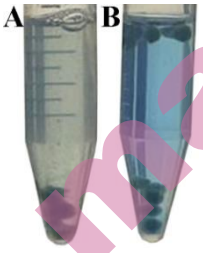


Fig 3. Alginate beads with encapsulated C-PC incubated at pH 1.35 (**A**) and 2.75 (**B**) and subsequently placed in 50 mM phosphate buffer at pH 7.

These results demonstrate that alginate immobilization can provide partial structural stabilization to C-PC under acidic conditions. This is noteworthy because soluble C-PC is unstable at pH levels below 5 and tends to aggregate.[28]

Interestingly, another study investigating C-PC encapsulation into alginate beads reported stability at pH 7, which we could not replicate due to significant leakage of C-PC.[22] The reason for this discrepancy remains unclear, especially since the preparation of alginate beads in both studies was almost identical. Possible factors could include differences in the starting materials (C-PC and alginate) or variations in the initial pH encapsulation and buffer compositions, which were not fully detailed in the other study.

The pH conditions for fabricating alginate beads play a critical role, as alginate is a pH-sensitive polymer. At higher pH values, alginate swells due to the numerous negatively charged carboxyl groups within its structure. Conversely, at lower pH values, these groups become protonated, causing the alginate gels to shrink. Since C-PC is physically entrapped within alginate gel formed by $Ca^{2+}$ cross-linking, the porosity of the alginate gel significantly impacts the system's stability. Additionally, C-PC's structural characteristics are influenced by pH. At higher pH, C-PC tends to dissociate from hexamers into trimers, while at lower pH, it adopts higher oligomeric states and tends to aggregate.[29] The higher oligomeric state of C-PC and the partial shrinkage of alginate likely prevented C-PC leakage from beads formed at pH 4 and lower.

*Structural stabilization of C-PC by alginate immobilization*

The stabilization of C-PC entrapped in alginate beads was tested under three different experiments: increased temperature, high pressure, and prolonged storage. Tested beads were incubated in 50 mM acetate buffer, pH 4.

The appearance of alginate beads with encapsulated C-PC remained unchanged after thermal treatment (Fig. 4A). Moreover, spectral analysis of C-PC released after incubating alginate beads at 60°C for 30 min showed a significant increase in C-PC's thermal stability under these conditions (Fig. 4B).

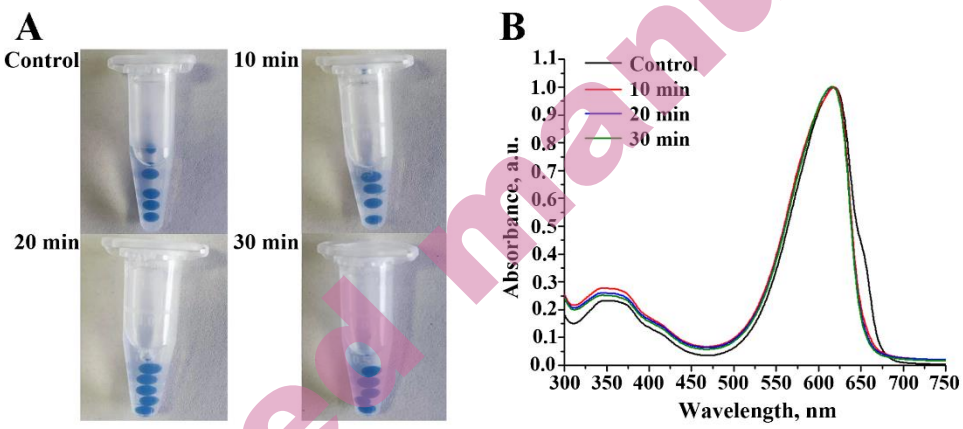


Fig 4. Alginate beads with encapsulated C-PC during thermal treatment at 60°C, photographed at 10, 20, and 30 min and after 30 min without thermal treatment, control sample (**A**), and absorbance spectra of control C-PC sample and C-PC samples after thermal treatment (**B**).

The $A_{620}/A_{280}$ absorbance ratio remained nearly the same, and the spectral shape showed minimal alteration compared to the non-heated control sample. The slight increase in absorbance around 350 nm suggests that the chromophore's conformation remained virtually unchanged during the incubation. The melting temperature ($T_m$) of C-PC is reported to range from 50 to 60°C, [30,31], and it depends on several factors, some of which are the purity and pH of the solution. Increased purity of C-PC reduces its thermal stability,[31] while its stability is greater at pH 5 compared to neutral and alkaline conditions.[32] At higher temperatures, C-PC undergoes structural degradation, destabilizing the chromophore and altering its color properties.[33]

Our findings suggest that the simple physical entrapment of C-PC within alginate beads provides both pH and thermal stability, thus expanding the potential industrial applications of C-PC. Two previous studies have also investigated the stability of encapsulated C-PC in alginate beads. One study dried the beads at 4°C and incubated them for three days at 35, 45, and 55°C. C-PC's stability was evaluated using the DPPH antioxidant test, concluding that alginate

immobilization enhanced thermal stability.[24] However, this study did not directly assess C-PC's structural characteristics, so its conclusions are limited. In another study, the authors incubated alginate beads with encapsulated C-PC at different temperatures, demonstrating similar stabilization at 60°C after 30 min by recording absorbance spectra in the visible range.[22] However, the pH conditions of their experiment were unclear.

In our study, we analyzed the structural characteristics of encapsulated C-PC by recording absorbance spectra from 300 to 750 nm, offering more detailed insights into C-PC and attached PCB behavior under specific treatments. During C-PC denaturation, PCB undergoes a conformational shift from linear to cyclic, evidenced by an increase in absorbance at 360 nm and a corresponding decrease at 620 nm.[34]

High-pressure (HP) food treatment is an environmentally friendly alternative to thermal treatment, offering superior preservation of nutritive components in treated foods.[35] In this study, alginate beads containing C-PC were subjected to high-pressure treatment at 4,500 bar for 30 min, resulting in a visible color change from blue to turquoise. This suggests that the encapsulated C-PC underwent structural alterations (Fig. 5A), a conclusion supported by the recorded absorbance spectra (Fig. 5B).

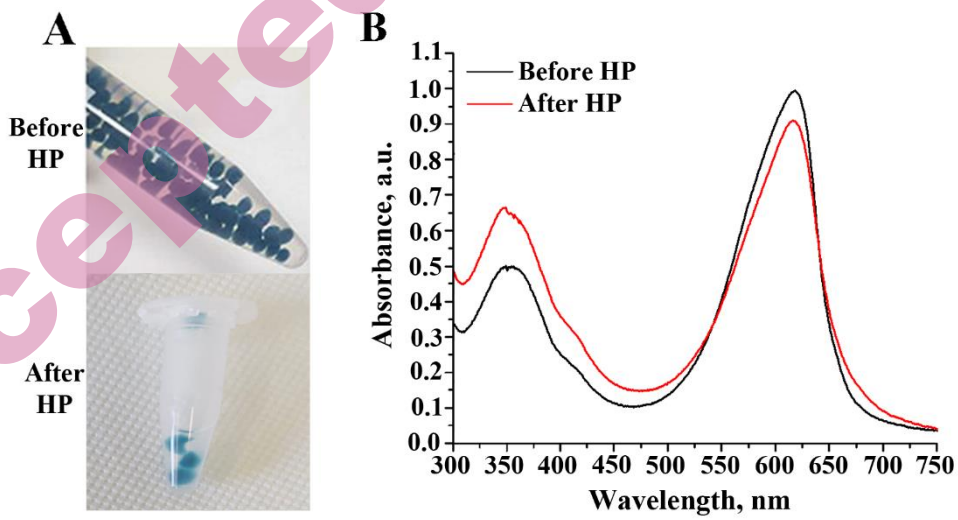


Fig 5. Alginate beads with encapsulated C-PC photographed before and after HP treatment (**A**), and C-PC absorbance spectra recorded before and after HP treatment (**B**).

Absorbance at 620 nm slightly decreased, while there was a parallel increase in absorbance around 350 nm. Additionally, the baseline of the post-treatment C-PC spectra showed a continual increase from higher to lower wavelengths. These

spectral changes indicate partial denaturation of C-PC, with the PCB chromophore transitioning from a linear to a more cyclic conformation. Similar findings were reported when HP treatment was used to extract C-PC from *Spirulina* biomass, where C-PC absorbance at 620 nm significantly decreased under 4,000 and 6,000 bar after only 3.5 min.[36] Other studies indicate that, at pH 7, C-PC's color is better preserved during HP treatment than during thermal treatment, even when C-PC is in solution.[37,38]

It is important to note that, in our study, HP treatment was conducted at pH 4, as this pH prevents leakage of encapsulated C-PC. Given C-PC's lower stability at this pH and the prolonged duration of HP treatment, our results imply that alginate encapsulation provides substantial stabilization for C-PC during HP processing.

Interestingly, after thermal treatment, the alginate beads appeared more compact (less swollen) than after pressure treatment. This could be due to the increased storage modulus of calcium-alginate gels at higher temperatures, which suggests greater gel stiffness.[39] In contrast, the electrostatic interactions that dominate alginate gels made by $Ca^{2+}$ may be disrupted under HP conditions. The pressure release could induce gel network restructuring, leading to a higher degree of swelling compared to the beads before pressure treatment.

*Storage stability of C-PC encapsulated in alginate beads*

To test storage stability, alginate beads with encapsulated C-PC were placed in a glass beaker without buffer and left to dry at 4°C for one month. After a month, the beads appeared flattened and adhered to the bottom of the beaker, with a dark blue appearance. Upon adding phosphate buffer (pH 7), the beads quickly swelled, releasing the encapsulated C-PC. This rapid release may be due to structural damage and surface cracks in the alginate caused by drying at low temperatures.[24]

The absorbance spectrum of the released C-PC was nearly identical to that of the control sample (Fig. 6), with only a minor increase in absorbance around 350 nm, indicating that the structure of the encapsulated C-PC remained largely intact during prolonged storage. These results suggest that in addition to freeze-drying,[20] alginate encapsulation is effective for stabilizing C-PC when dried at 4°C for at least a month.

*Mercury removal by alginate beads containing C-PC*

C-PC has been shown to bind mercury ions with high affinity in the pH range from 5 to 10.[11] Considering that, in our study, alginate encapsulation of C-PC was most efficient at pH 4, we tested the potential for mercury removal by immobilized C-PC at this pH. The fluorescence intensity of free C-PC in solution decreased as increasing concentrations of $Hg^{2+}$ were added, indicating an interaction between C-PC and $Hg^{2+}$ (Fig. 7A). The affinity constant was calculated from the obtained fluorescence spectra, yielding a value of $4 \times 10^6\ M^{-1}$ (Fig. 7B), confirming that free C-PC binds $Hg^{2+}$ with high affinity. When encapsulated C-PC was incubated with

$Hg^{2+}$ solution for two days at room temperature with constant shaking, more than 97 % of the mercury was removed from the solution (Fig. 7C). Control samples consisting of empty alginate beads had almost no effect on mercury binding, demonstrating that the presence of C-PC is crucial for mercury removal.

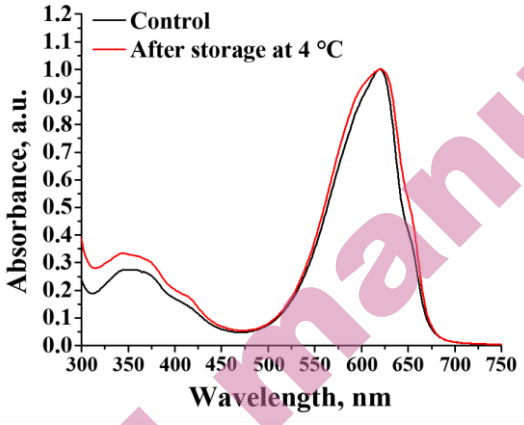


Fig 6. Absorbance spectra of encapsulated C-PC obtained before (control sample) and after incubation of dried alginate beads at 4°C for one month.

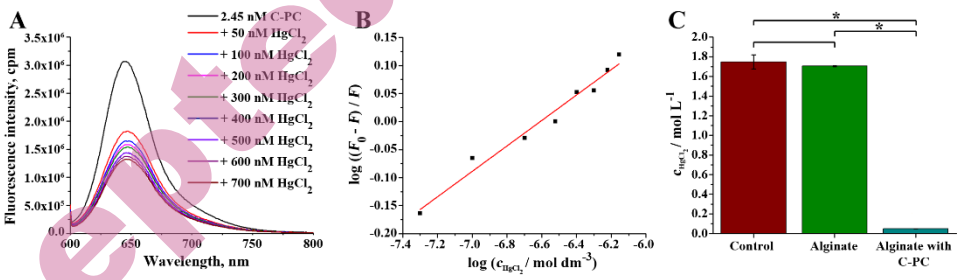


Fig 7. Fluorescence spectra of C-PC in the presence of increasing concentrations of $Hg^{2+}$ (**A**). Plot used to calculate the affinity constant ($K_a$) from Eq. 1 (**B**). Concentration of $Hg^{2+}$ in solution without alginate beads (Control), after incubation with empty alginate beads, and with alginate beads containing encapsulated C-PC (**C**). Statistically significant differences between groups, $p < 0.05$, are marked with the '*' symbol.

In a previous study, C-PC immobilized onto the surface of chitosan was also effective for mercury removal, with binding occurring within 1 h.[25] Since alginate encapsulation entraps C-PC inside the alginate matrix, without direct exposure to metal ions, $Hg^{2+}$ must diffuse into the alginate beads for binding to occur, which prolonged the process. This is why we chose a 2 day-incubation period in this study. Additionally, we tested beads made with 0.5% alginate, which created a more porous structure and sped up the diffusion process.

Adsorption as a method for heavy metals removal is widely studied due to its operational simplicity, reusability potential, and efficiency.[40] Choosing an efficient, low-cost system is critical. Thus, only partial purification of C-PC was performed in this study. Further purification of C-PC could enhance the system's mercury removal efficiency, though it would also significantly increase the cost of the encapsulated material. While using encapsulated C-PC in alginate for heavy metal removal shows promise, further research is needed to optimize this approach.

## CONCLUSION

In this study, the immobilization of C-PC through alginate entrapment was optimized. The primary factor controlling C-PC leakage from alginate beads was pH, with pH of 4 being the most favorable, showing almost no C-PC leakage. Under these conditions, the beads retained an intense blue color, indicating that the structure of C-PC is preserved, as confirmed by absorption spectroscopy. Alginate immobilization provided significant stabilization for C-PC during both high-temperature and high-pressure treatments. Additionally, stabilization at low pH was observed, as beads prepared in acidic conditions released blue C-PC when placed in a neutral buffer, suggesting the preservation of C-PC structure to some extent. The shelf life of immobilized C-PC was also enhanced, and alginate/C-PC beads demonstrated efficient mercury ion removal at pH 4, achieving a 97% removal rate under tested conditions. The results suggest that alginate is an effective medium for immobilizing C-PC at pH 4, enhancing its stability and expanding its potential applications in the food industry and bioremediation efforts.

*Acknowledgments:* This work was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia, under contract numbers 451-03-66/2024-03/200168 (Faculty of Chemistry) and 451-03-66/2024-03/200026 (Institute of Chemistry, Technology, and Metallurgy). This study was also partially supported (high-pressure experiments) by the Alliance of International Science Organizations (ANSO), Project No. ANSO-CR-PP-2021-01.

## И З В О Д

### ОПТИМИЗАЦИЈА АЛГИНАТНЕ ИМОБИЛИЗАЦИЈЕ Ц-ФИКОЦИЈАНИНА ДОБИЈЕНОГ ИЗ ХРАНЕ: СТРУКТУРНА И ФУНКЦИОНАЛНА КАРАКТЕРИЗАЦИЈА

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Ц-фикоцијанин (Ц-ПЦ) представља значајну компоненту биомасе цијанобактерије *Arthrospira platensis* (Спирулина). Осим своје нутритивне вредности, овај протеин испољава бројне корисне биолошке активности. Ковалентно везана хромофора, фикоцијанобилин, даје Ц-ПЦ-у плаву боју, што омогућава његову употребу као природне боје за храну. Поред тога, фикоцијанобилин испољава различита биоактивна својства, укључујући активности везивања метала. Кључни недостатак шире индустријске примене Ц-ПЦ-а је његова лоша стабилност. Недавно су алтернативне формулације хране које користе природне полимере као носаче и активне компоненте привукле значајну научну пажњу. Овај рад описује оптимизоване услове за имобилизацију Ц-ПЦ-а коришћењем алгината. Структурна стабилизација имобилизованог Ц-ПЦ-а је испитана под високом температуром (60°C) и високим притиском (450 MPa). Стабилност складиштења имобилизованог Ц-ПЦ-а у осушеним куглицама алгината је тестирана држањем узорака на 4°C током једног месеца. Такође је испитана потенцијална примена имобилизованог Ц-ПЦ-а за уклањање јона живе. Имобилизација алгината се показала ефикасном у стабилизацији Ц-ПЦ-а, значајно чувајући његову структуру током дужег складиштења, термичке обраде и излагања високом притиску. У тестираним условима, 97% $Hg^{2+}$ јона је уклоњено имобилизованим Ц-ПЦ-ом. Свеобухватно, ова студија је оптимизовала процедуру за побољшање стабилности Ц-ПЦ-а алгинатном имобилизацијом и проширила његову потенцијалну примену у индустрији хране и биоремедијације.

(Примљено 25. октобра 2024; ревидирано 7. јануара 2025; прихваћено 27. јануара 2025.)

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